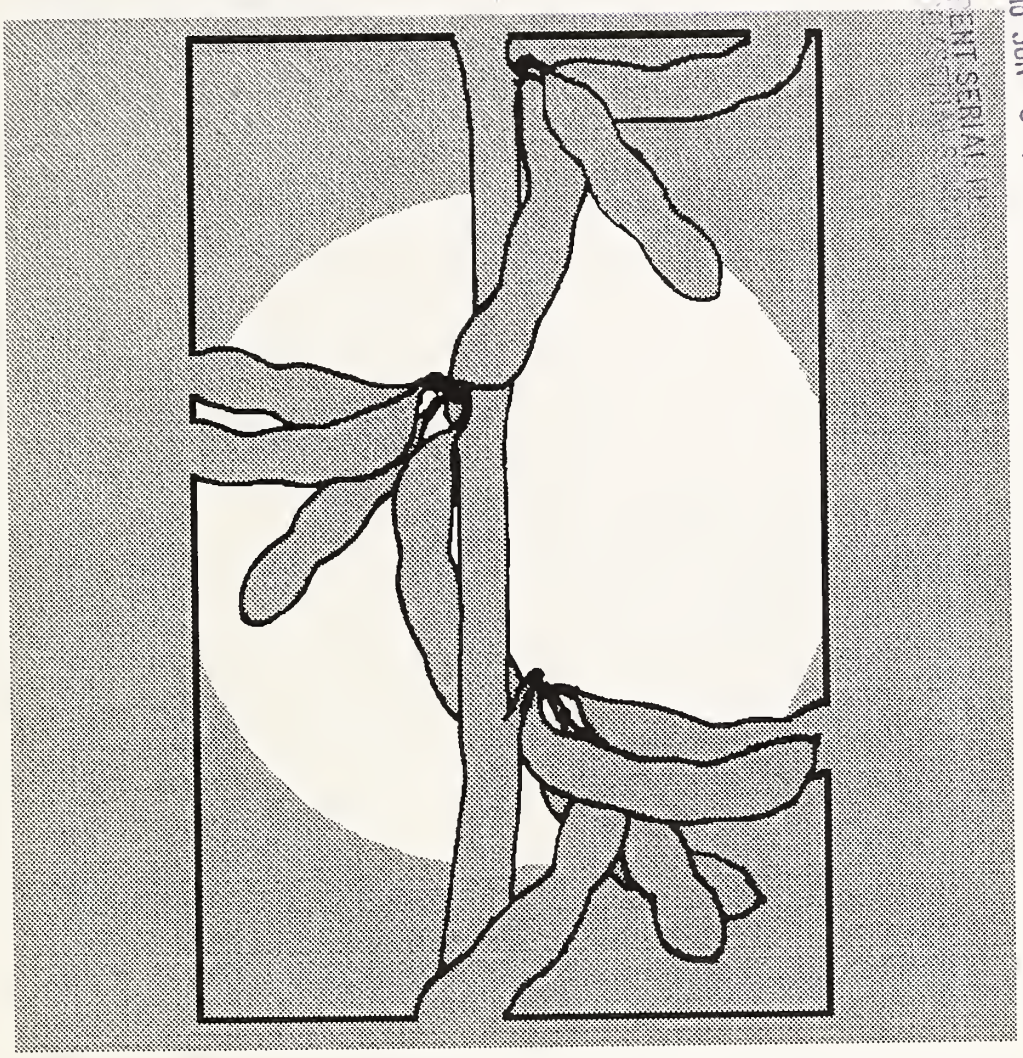
Soybean Genetics Newsletter



Volume 25 May 1998

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Foreword

Welcome to the Silver Anniversary volume of the Soybean Genetics Newsletter. In volume 1 1974, we state that the Newsletter 'will serve as a means of communication at the international level'. As you browse the Table of Contents of this volume note that the international contribution is impressive and compliments the U.S. contributions. Thank you for 25 productive years.

Please note the announcement of World Soybean research Conference VI.

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Soybean Genetics Newsletter Homepage
http://www.public.iastate.edu/~rpalmer/Soybean Genetics.html

You can now visit our homepage. Browse the table of contents and query of authors and articles for volume 1 (1974) to current volume. The home page is under continuing construction, and is updated bimonthly.

WORLD SOYBEAN RESEARCH CONFERENCE VI
CHICAGO, ILLINOIS, USA
AUGUST 4-7, 1999

The Sixth World Soybean Research Conference (WSRC VI) will be held in Chicago, Illinois, USA, August 4-7, 1999. The meetings will take place at the Sheraton Chicago Hotel and Towers on the shore of Lake Michigan. The University of Illinois is the host organization for the conference. A program committee is developing the program which will consist of symposia and contributed paper and poster sessions in the following areas: Crop Improvement (Genetic Resources, Breeding, Physiology, and Biotechnology), Crop Production (Crop Management, Soil and Water Management, and Pest Management), Processing (Solvent Extraction, Non-chemical), and Utilization (Animal Feed, Food and Health, and Industrial).

The WSRC VI will be held in conjunction with the Global Soy Forum 99 (GSF) which is a worldwide meeting of the soybean industry organized by Soybean Research and Development Council and the National Soybean Research Laboratory. The goal of GSF is to assemble producers, researchers, industry leaders, policy makers, and consumers from around the world to celebrate the past successes of the soybean industry and to position the industry to respond to the challenges and opportunities of the 21st century. The registration fee for the WSRC will also cover participation in the events planned by the GSF99.

A call for papers is being prepared for release. To receive the announcement or to register, visit the WSRC VI web site at http://www.gsf99.uiuc.edu/wsrc.html or contact Dr. Harold Kauffman, Chairman of WSRC VI at:

WSRC VI
National Soybean Research Laboratory
1101 W. Peabody
Urbana, Illinois 61801
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Email: hkauffma@uiuc.edu.
Phone: (217-244-7384)
FAX: (217-244-1707)

USDA Soybean Germplasm Collection Report -- 1997

February 1998

In 1997, a total of 8,522 seed lots were distributed from the USDA Soybean Germplasm Collection in response to 335 requests from 181 individuals. Domestically, 281 orders with a total 7,936 seed packets representing 5,828 accessions were sent to 138 researchers from 31 states. There were 586 seed packets of 494 accessions in 58 orders sent to 42 scientists in 20 foreign countries. We also sent seeds of 586 accessions to the National Seed Storage Lab for backup storage.

Plots planted for seed replacement of *G. max* accessions in 1997 consisted of 1432 four-row plots planted at three locations: 1218 at Urbana, Illinois; 175 at Stoneville, Mississippi; and 39 plots at Isabela, Puerto Rico. Seed replacement plots for *G. soja* accessions were planted in both Stoneville (42) and Urbana (10).

In 1997, we grew 436 new foreign *G. max* accessions for the first time. These originated from China (406), South Korea (15) and Ukraine (15). Most of the Chinese accessions (346) came from far southern China and were grown in Puerto Rico during the winter of 1996-97. The remaining were planted at Stoneville (10) and Urbana (80). Seventeen *G. soja* accessions from the Chinese provinces of Heilongjiang (3), Shanxi (6), and Shandong (6) and Zhejiang (2) were grown for the first time in 1997. We also received seeds of 10 domestic cultivars, 15 germplasm releases, and 1 genetic type.

Primarily due to the introductions that came in 1996 from the third set in the Chinese germplasm exchange project, there were over 3300 pureline plots planted in 1997. Most were planted at Stoneville (2733) with 550 at Urbana, and 56 in Puerto Rico. The pureline rows from most of the new introductions grown in Puerto Rico during the winter of 1996-97 were planted at Stoneville in the summer of 1997. Processing these new accessions is only partially completed but to date 780 new accessions have been added to the collection from the 1997 harvest. The new accessions are from China (643), Japan (86), South Korea (50), and the Philippines (1). Finding the right environment for seed production for some of the accessions from southern China has been difficult and the pureline process for 65 original seed lots has not been completed. Accessions received from Anhui, Fujian, Hunan, Sichuan, Jiangxi, Guangxi, Yunan, and Guizhou in southern China ranged from maturity group II to maturity group X.

The second year of the general field evaluation of late maturity accessions received from China in the first and second germplasm exchanges was planted at Stoneville in 1997. That planting consisted of 1235 lines, an increase of 190 over the 1996 planting. Some accessions tentatively placed in maturity groups IV and IX were grown to help determine the proper maturity classification of these lines. In addition, there were 184 recent U.S. southern named cultivars planted to check pubescence characters. In previous evaluations, we have learned that there is more variation in pubescence type and orientation among U.S. southern cultivars than among U.S. northern cultivars. With very few exceptions all U.S. northern cultivars have erect pubescence with normal density. For the U.S. southern cultivars, we are finding some cultivars with semi-sparse density and many cultivars with appressed or semi-appressed pubescence.

As of December 31, 1997, the Collection contained the following entries:

Subcollection	Entries
Introduced *G. max*	14,358
G. soja	1,103
Germplasm releases	141
Modern cultivars	408
Old cultivars	208
Private cultivars	31

Subcollection	Entries
All isolines	579
Williams	(100)
Clark	(295)
Harosoy	(136)
Other	(48)
Genetic types	150
Total	16,978

The following information was taken from the annual report of Dr. Ted Hymowitz for the specific cooperative agreement "Management of the USDA Perennial *Glycine* Germplasm Collection". The current inventory of the perennial *Glycine* consists of 1032 accessions of 16 species. Of these, 851 accessions in 13 species are available for distribution and 774 accessions in 12 species are stored at the National Seed Storage Laboratory in Ft. Collins. During the 1997 report year that ended on September 30, 1997, 521 seed packets were sent in response to 23 requests from 5 states and 1 foreign country.

J.L. Hill and R.L. Nelson
USDA-Agricultural Research Service
Soybean Germplasm Collection
1101 W. Peabody Drive
Urbana, Illinois 61801

1998 Soybean Crop Germplasm Committee Meeting

The Soybean Germplasm Committee held its annual meeting February 23, 1998 in conjunction with the Soybean Breeder's Workshop at St. Louis, MO. In attendance were committee members John Hicks, Bob Freestone, Earl Hendrix, Elroy Cober, Randall Nelson, Terry Niblack, Jerry Hill, Tom Kilen, Dan Phillips, Michael May, Jim Orf, Richard Wilson, Emerson Shipe, and guests Tommy Carter, Mark Bohning, Grover Shannon, Chris Tinius, and R. Rossi. Chair, Emerson Shipe, called the meeting to order at 8:00 a.m. New members joining the committee for a 3-year term are Howard Gabe, Dan Phillips, and Richard Wilson. Emerson Shipe distributed copies of a document which describes the function, duties, and responsibilities of crop germplasm committees.

An agenda was sent to the committee members prior to the meeting naming members to the four subcommittees and listing topics for discussion. The committee broke into subcommittees until 9:30 a.m.

Acquisition (Orf, Chair; Ashley, Hicks, Nelson, Gabe)

The committee passed a motion to continue to include all publicly developed varieties in the germplasm collection. The developer will have the option to not have the variety distributed until a later date (i.e. it will be stored but not distributed) as determined by the originator. PVP varieties will be distributed with the originator's permission.

The following discontinued private varieties were accepted into the germplasm collection: S09-90, B152, S1346, S23-03, S42-30, Coker 237, and Coker Hampton from Novartis Seeds and CX174 from Dekalb Genetics.

A collection trip to Vietnam is planned for late summer 1998 by R. Nelson and T. Hymowitz. New accessions continue to be obtained from China under the current four-year exchange agreement that began in 1996. The G. soja collection is quite small compared to the G. max collection and additional G. soja accessions are needed from all places where this species is native: China, Russia, Japan and the Korean peninsula. There are no immediate plans for acquisition or collection. Opportunities to collect or acquire G. max germplasm from areas of ancient cultivation not well represented in the Collection are being explored.

Evaluation, Crop Vulnerability (Phillips, Chair; Niblack, Hendrix, Cober, All)

The topic of maintenance and distribution of pathogens critical to the evaluation and screening of soybean germplasm continues to discussed. A committee is being formed to develop a proposal for funding of a workshop in order to develop a long-term solution. Dan Phillips will solicit additional input and organize this group.

Enhancement (Freestone, Chair; Buxton, Shipe, May, Wilson)

A wide-ranging discussion was held concerning the need for timely release of publicly developed germplasm in order to maintain genetic diversity in breeding programs. The committee recomended that USDA agressively pursue "material transfer agreements" for germplasm release. To facilitate this the committee will: (A) draft a white paper on germplasm release; (B) alert the ASTA research committee about this topic for possible inclusion in the 1998 or 1999 program; (C) alert ESCOP about this concern so the topic will be discussed in their meetings. The committee passed a motion to recommend reinforcement of current soybean germplasm enhancement programs in various USDA strategic plans that are now being developed.
A germplasm/variety release notice draft for public releases has been developed and is being refined. It is a one-page form with essential characteristics listed in table form that will be more readable and in a standard format. The Committee passed a motion recommending that the planning committee of the Soybean Breeders Workshop include a "Public Release" topic in the program each year. This would be a summary that lists newly released public varieties and germplasm for the previous year.

The Committee recommended that current marker technologies be used to better define the gene pool structure of the germplasm collection.

Operations (Kilen, Chair; Delannay, Hill, Bohning)

Data from evaluation of new Chinese material continues to be entered in the GRIN system. The germplasm collection continues to grow and work involved in entering new accessions and renewing old accessions

increases each year, yet staff and budgets have not increased. This is a problem. PC GRIN is now available for use by researchers in other countries. It will be first used in Latin America. Dr. Peter K. Bretting has recently been named Research Leader/Coordinator for USDA Plant Genetic Resources.

Other Business

Jerry Hill distributed the 1997 Soybean Germplasm Collection Report. In 1997, 8,522 seed lots from 335 requests of 181 individuals were distributed. Requests came from 138 researchers in 31 states, 6 from Canada, and 36 researchers in 19 foreign countries. There are now 16,978 accessions in the G. max and G. soja collection.

Mark Bohning distributed a progress report on GRIN. Pertinent points from this report are found under the Operations subcommittee report.

Tommy Carter presented information which indicates that the rate of breeding progress for yield (in bu./ac/yr.) has increased in the Midwest and declined in the South when comparing the 1980's vs. the 1990's. These trends are based on performance variety trials in 10 states. The increased progress in the Midwest, although substantial, has not kept pace with the increased breeding effort, suggesting that an increment of yield progress is more difficult to obtain now than in the early 1980's. These trends may be a result of declining genetic diversity in applied breeding programs. He plans to publish these findings in the Soybean Genetics Newsletter.

Appreciation was expressed to retiring members John Hicks and Doyle Ashley. Jim Orf was elected Chair for 1998. Terry Niblack was elected Vice-chair. Meeting adjourned at 11:55 a.m.

E. R. Shipe, Chair
J. Orf, Vice-chair

Soybean Crop Germplasm Committee Members

March 11, 1998

MEMBER	AREA OF REPRESENTATION	TERM EXPIRES
All, John University of Georgia Department of Entomology Athens, GA 30602-7503 Phone: 706/542-7589 Fax: 706/542-2279 E-Mail: jall@bugs.ent.uga.edu	Entomology	2000
Wilson, Richard USDA-ARS 4114 Williams Hall 100 Derieux St., Box 7620 NC State University Raleigh, NC 27695-7620 Phone: 919-515-3171 Fax: 919-515-7959 E-mail: rwilson@cropserv1.cropsci.ncsu.edu	Physiologist	2001
Buxton, Dwayne USDA-ARS-NPS Bldg. 005, BARC-W Beltsville, MD 20705 Phone: 301/504-5289 Fax: 301/504-5467 E-mail: DRB@ars.usda.gov	National Program Staff	Ex Officio
Cober, Elroy Agriculture-Canada Bldg. 110 Central Exp. Farm Ottawa, Ontario K1A 0C6 Canada Phone: 613/759-1610 Fax: 613/759-6597 E-mail: coberer@em.agr.ca	Canadian Representative	2000
Delannay, Xavier Monsanto Co. 700 Chesterfield Pkwy. W. St. Louis, MO 63198 Phone: 314/537-6611 Fax: 314/537-6759 E-mail: xavier.delannay@monsanto.com	Cytogenetics & Molecular Genetics	2000

MEMBER	AREA OF REPRESENTATION	TERM EXPIRES
Freestone, Robert Pioneer Hi-Bred 3261 W. Airline Highway Waterloo, IA 50703 Phone: 319/234-0335 Fax: 319/233-8650 E-mail: freestone@phibred.com	Private Breeding, North	1999
Hendrix, Earl 500 Chisholm Road Raeford, NC 28376 Phone: 910/875-0229 Fax: 910/875-3693 E-mail: hendrix-farms@worldnet.att.net	Chair, U.S.B. Production Committee	Ex Officio
Gabe, Howard Novartis P.O. Box 729, Hwy. 158 E. Bay, AR 72411 Phone: 870-483-7691 Fax: 870-483-7179 E-mail: Howard.gabe@seeds.novartis.com	Private Breeding, South	2001
Hill, Jerry USDA-ARS University of Illinois 1101 West Peabody Urbana, IL 61801 Phone: 217/244-4345 Fax: 217/333-4639 E-Mail: j-hill26@uiuc.edu	USDA Germplasm Collection	Ex Officio
Kilen, Thomas C. Soybean Production Research USDA-ARS P.O. Box 196 Stoneville, MS 38776 Phone: 601/686-3125 Fax: 601/686-3140 E-Mail: tkilen@ag.gov	USDA Germplasm Collection	Ex Officio
May, Michael United Soybean Board 540 Maryville Centre Dr., Suite LL5 St. Louis, MO 63141 Phone: 314/579-1580 Fax: 314/579-1599	Prod. Project Administrator, U.S.B.	Ex Officio

E-Mail: mike_may@sba.com

MEMBER	AREA OF REPRESENTATION	TERM EXPIRES
Nelson, Randall University of Illinois Dept. of Crop Sciences, NSRL, USDA 1101 West Peabody Urbana, IL 61801 Phone: 217/244-4346 Fax: 217/333-4639 E-Mail: rlnelson@uiuc.edu	USDA Germplasm Collection	Ex Officio
Niblack, Terry, Vice-Chair Dept. of Plant Pathology 108 Waters Hall University of Missouri Columbia, MO 65211 Phone: 573/882-7333 or 2432 Fax: 573/882-0588 E-Mail: niblack@psu.missouri.edu niblack@showme.missouri.edu	Nematology	1999
Orf, Jim, Chair Dept. of Agronomy & Plant Genetics University of Minnesota St. Paul, MN 55108 Phone: 612/625-8275 Fax: 612/625-1268 E-Mail: orfxx001@maroon.tc.umn.edu	Public Breeding, North	2000
Phillips, Daniel Dept. of Plant Pathology University of Georgia 1109 Experiment Station Griffin, GA 30223 Phone: 770/412-4009 Fax: 770/228-7202 E-Mail: dphilli@gaes.griffin.peachnet.edu	Plant Pathology	2001
Shipe, Emerson Dept. of Crop & Soil Environmental Science Clemson University Box 340359 Clemson, SC 29634-0359 Phone: 864/656-3524 Fax: 864/656-3443 E-mail: eshipe@clemson.edu	Public Breeding, South	1999

Soybean Genetics Committee Report

The Soybean Genetics Committee met February 23, 1998 at the Sheraton Inn, St. Louis, MO in conjunction with the Soybean Breeder's Workshop. Committee members attending the meeting were P. R. Arelli, G. R. Buss, P. B. Cregan, B. W. Diers, R. L. Nelson, T. W. Pfeiffer, and S. K. St. Martin. P. B. Cregan and T. W. Pfeiffer had been elected by mail ballot to serve a three-year term on the committee. Brian Diers was elected chair for the year ending February, 1999. Current committee members and February expiration dates for their terms on the committee are:

Dr. B. Diers (2000) (Chair)
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Fax: (517) 353-3955
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Dr. G.R. Buss (1999)
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E-mail: gbuss@vt.edu

Dr. P.B. Cregan (2001)
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BARC-West
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Columbus, OH 74078
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Procedure

As in the past, manuscripts concerning gene symbols and linkages should be sent to the Chairperson of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Authors may submit unpolished (but comprehensible) manuscripts for review which should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of four weeks to return the reviewed manuscript to the Chairperson (who will then return it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or have not phoned in their comments), a phone call will be

made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairperson of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols

Seed samples containing named genes, and especially those from induced mutants, variants from heterogeneous populations, or from transgenic changes should be deposited in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups. A form for this purpose is on page 19 of this volume.

Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

New Business

There was discussion on what manuscripts should be reviewed by the Soybean Genetics Committee. Manuscripts have been submitted to the committee only for a review of genetic interpretation and not for gene symbol or linkage assignments. The committee concluded only manuscripts that require a review for gene symbols or linkage assignments will be reviewed by the committee. Manuscripts submitted only for a review of genetic interpretation would be returned to the author without being reviewed.

The committee discussed the Soybean Genetics Newsletter and the poor funding Dr. Reid Palmer receives to edit and publish the newsletter. The committee expressed their appreciation to Dr. Palmer for the work he puts into publishing the newsletter. To reduce publishing costs, the committee recommends that within two years the newsletter quits accepting hard copy article submissions and only accepts articles submitted by E-mail or computer diskette. The committee also recommends that the newsletter move to an internet based publishing format.

The committee discussed the naming of quantitative trait loci (QTL). There was a consensus that a naming system should be established. This system would be used to catalog soybean QTL that have been published. After QTL are published, these would be named and cataloged on the SoyBase database program on the internet.

Gene Symbols/Linkages assigned March 1997 - February 1998.

Date	Author	Trait	Gene/Linkage
4-21-1997	Devine, T.	Linkage Group 14	*y17* - *pb* 27 map units
5-29-97	Cober, E. Ablett, G. Buzzell, R. Luzzi, B. Poysa, V. Voldeng, H.	Imperfect yellow hilum color	Conditioned by *T* and *I* alleles
5-23-97	Chung, J. Staswick, P. Graef, G. Wysong, D. Specht, J.	Disease lesion mimic	*dlm* and *DLM*
10-12-97	Stojsin, D. Luzzi, B Ablett, G. Tanner, J.	Low linolenic acid concentation	*fan-b*

Date	Author	Trait	Gene/Linkage
11-13-97	Cober, E. Voldeng, H.	Photoperiod sensitivity	*E7* and *e7*
2-3-98	Chen, X. Imsande, J. Palmer, R.	Yellow foliage	Drop symbol *y15* from T234. New symbols: *y20* (Ames 17), *y20* (Ames 18), *y20* (Ames 19), *y20* (Ames 20), *y20* (Ames 21), *y20* (Ames 22), *y20* (Ames 23)
		Mitochondrial malate dehydrogenase 1 null	*Mdh1-n* (Ames 18), *Mdh1-n* (Ames 19), *Mdh1-n* (Ames 20), *Mdh1-n* (Ames 21), *Mdh1-n* (Ames 22), *Mdh1-n* (Ames 23), *Mdh1-n* (Ames 24), *Mdh1-n* (Ames 25)
2-17-98	Palmer, R. Horner, H.	Genic male-sterile, female-sterile	*st8* and *ST8*

Guidelines on the Evidence Necessary for the Assignment of Gene Symbols

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion. Gene symbols will only be approved in cases where the relevant (germplasm) material is made available for subsequent allelism testing.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the F_2 generation and here called the hypothesis generation.

2. A second generation with a pedigree trace to the first generation, is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually F_3) or progeny of a testcross (F_1 x recessive homozygote).

3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Testcross data are not suitable for this purpose.

4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes. If appropriate allelism tests are not included in a manuscript, the committee will request such information from the researcher. Molecular linkages can also be used to demonstrate that the allelism test conducted is the only one needed.

5. Identification of cytoplasmic factors requires reciprocal crosses between parents differing in the trait of interest. Since these factors are transmitted through the cytoplasm, the trait is expected to be associated only with the maternal parent in the F_1 and succeeding generations. Maternal effects need to be distinguished from cytoplasmic effects by using reciprocal F_1 and F_2 data.

6. Conclusive evidence for cytoplasmic factors should rule out self pollinations and nongenetic factors associated with the maternal parent. Selecting parents for reciprocal crosses that differ in nuclear genetic traits (e.g., flower or pubescence color) in addition to possible cytoplasmic traits will provide evidence of cross- rather than self-pollinations by observed segregation for the nuclear genetic trait in succeeding generations.

7. Inheritance patterns in a hypothesis generation (F_2) and a confirming generation (F_3) are absolute requirements for differentiating between cytoplasmic factors and nuclear genetic traits.

8. Follow the guidelines (Rules for Genetic Symbols) published in the Soybean Genetics Newsletter to assign the symbol.

9. Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address). Please indicate in unequivocal terms your willingness to provide seed for allelism tests requested by researchers discovering genes with a similar phenotype. This does not restrict your asking for a signature on a Material Transfer Agreement.

10. If the line in which the new gene occurs in not already in the USDA Germplasm Collection, you are strongly encouraged to send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see the current Soybean Genetics Newsletter for name and address).

References

Allard, R. W. 1956. Formulas and tables to facilitate the calculation of recombinational values in heredity. Hilgardia 24:235-278.

Hanson, W. D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.

Immer, F. R. 1930. Formulae and tables for calculating linkage intensities. Genetics 15:81-98.

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Mather, K. 1951. The measurement of linkage in heredity. Methuen and Co., Ltd. London. John Wiley and Sons, Inc. New York.

Sedcole, J. R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

I. Gene Symbols

a. Gene symbols will not be assigned to traits for which no inheritance data are presented.

b. A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.

c. Genes that are allelic shall be symbolized with the same base letter(s) so that each genetic locus will be designated by a characteristic symbol base.

d. Gene pairs that govern the same phenotype (including duplicate, complementary or polymorphic genes) constitute multiple loci that should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1, Y_2, etc.) The numerals may be written on the same line as the base. (Example: *Y1*, *Y2*, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.

e. The first pair of alleles reported for a genetic locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: *Ab*, *ab*; *Ab* is allelic and dominant to *ab*.)

f. If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles, or those symbolized subsequently to the pair first published, shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: *R*, r^m, *r*.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (Example: *Rps1-b, Rps1-k*, and *Ap-a, Ap-b, Ap-c*.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

   If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should **NOT** be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. [Example: *ms1* (Tonica), or *ms1* (Ames 2).] This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

g. Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

h. The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clearcut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

i. An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

j. A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. [Example: *Rps?* (Harosoy) an allele in Harosoy at an unknown locus or *Ap-?* (T160) an unknown allele in T160 at the *Ap* locus.]

k. Plus (+) symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II. Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybean.

a. A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. [Example: Adh (alcohol dehydrogenase), Idh (isocitrate dehydrogenase).] The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.

b. The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.

c. Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III. Probe detected loci

The following guidelines are to be used for assigning locus names to probe-detected (RFLP) loci. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybean.

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix is followed by a string of letters and/or integers that identify the probe used to detect the locus by the originating laboratory. This probe-identifying string should be limited to no more than six characters. This string should be separated from the prefix by a hyphen. (Example: IaSU-B317).

c. The probe-identifying string is followed by the restriction endonuclease used in the restriction digest of the soybean genomic DNA that was probed. The following abbreviations for restriction enzymes are recommended: EcoRI = I, EcoRV = V, HinddIII = H,DraI = D, RsaI = R, Bc1I = B, TaqI = T. (Example: IaSU-B317I, IaSU-B317T).

d. Duplicate loci detected by the same probe should be identified with the same letter and integer base differentiated by integers (1, 2, 3, 4, etc.) consecutively assigned in the order of publication. These numerals are to be separated from the base string by a hyphen. Example:

IaSU-B3171-1, IaSU-B3171-2, etc.)

e. Upon publication of new RFLP loci, researchers are strongly encouraged to

1. make the probe identifying the locus/loci publicly available
2. make available the identity of the restriction endonuclease used to generate the mapped polymorphism
3. make available the identity of the genetic stock used to map the locus/loci
4. make available the molecular weights of the polymorphic fragments used to map the locus/loci

IV. Random Amplified Polymorphic DNA (RAPD) loci

The following guidelines are to be used in assigning names to loci that are mapped using RAPD technology. The system adopted here is that which is generally employed in other species in which RAPD loci have been mapped.

a. Locus designations should begin with a letter identifying the origin of the primer. (Example: Operon Technologies, O)

b. The origin of the primer is followed by the primer name. (Example: Primer number 14 from Operon Technologies kit A, OA14.)

c. The primer name is followed in subscript by the fragment size in base pairs of the amplified fragment that is being mapped. (Example: An 800 bp fragment amplified with Operon Technologies primer 14 from kit A, $OA14_{800}$).

V. Simple sequence repeat (SSR) or microsatellite loci

a. Locus designations should be prefixed with a modified postal state identifier and/or institution identifier that will minimize ambiguity from similarities in probe names. (Example: Iowa St. Univ., IaSU). The prefix shall not be necessary in publications except as needed to distinguish numbers that would, without the prefix, be identical.

b. The prefix should be followed by a string of letters that identify the core nucleotide repeat of the SSR followed by an identifying number. This string should be separated from the prefix by a hyphen and should not exceed eight characters. (Example: IaSU-at275, BARC-gata3412).

c. Upon publication of new SSR loci, researchers are strongly encouraged to

1. make available the oligonucleotide primer sequences required for amplification of the SSR
2. make available the identity of the genetic stock used to map the locus/loci.

VI. Linkage and Chromosome Symbols

a. Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b. Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of

aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv, inversions; and Tri, primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c. Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2 the second case, etc. The first published deficiency shall be symbolized as Def A, the Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

VII. Cytoplasmic Factor Symbols

a. Cytoplasmic factors shall be designated with one or more letters prefixed by *cyt-*. (Example: *cyt-G* indicates the cytoplasmic factor for maternal green cotyledons, *cyt-Y* indicates that for maternal yellow cotyledons.)

b. Designations for specific cytoplasmic factors following *cyt-*, shall follow the same format as for gene symbols. Base letters chosen to indicate apparent relationships among traits will have common initial letters for all loci in a related group of traits. Initial letters will be consistent with initial letters designating nuclear gene traits. (Example: *cyt-G* green seed embryo, *cyt-Y2* yellow leaves, becoming yellowish green.)

VIII. Priority and Validity of Symbols

a. A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b. In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

IX. Rule changes

a. These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date: _____ T number (assigned by curator) _____

Submitted by: _____

Address: _____

_____

_____

_____

_____

_____

_____

Return to:
R.L. Nelson, curator
USDA Soybean Germplasm Collection
Department of Agronomy
University of Illinois
1102 South Goodwin Avenue
Urbana, Illinois, 61801, U.S.A.

Strain Designation: _____

Genotype: _____

Phenotype: _____

_____

Parental Origin: _____

_____

_____

_____

When and where found and by whom: _____

_____

_____

_____

Description

Maturity Group _____ Stem termination _____

Pubescence color _____ Pubescence type and density _____

Seed coat luster and color _____ Hilum color _____

Flower color _____ other _____

Pod color _____ _____

Special instructions for growing or maintenance, if any: _____

_____

Literature References: _____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait. Please send relevant reprints to the curator.

Date seedlot received at Urbana: _____ Date T number assigned: _____

Unidad Integrada Balcarce
cc 272, (7620) Balcarce
Argentina

R. Carbone
J. Lúquez
M.E. Weilenmann de Tau
J.C.Suárez

Frequency of Recombination of Loci L_1 and L_2 that Encode for Presence of Lipoxygenase Enzymes in Soybean Seeds

Introduction

Soybean is the most important crop of Argentina. Most of the grain produced is processed and exported as oil and by products. Because Argentina exports oil, research is oriented to obtain genotypes improved in their quantity and quality of proteins and oil. Argentina was the principal exporter country of soybean in the world in 1996, with 1.694.061 tn. Oil soybean supplies 25% of requirements in fats and oils in the world, and 95% of this oil is used for food.

Lipoxygenase enzymes are responsible for the oxidation of unsaturated fatty acids (linoleic and linolenic) of soybean seed. This oxidation occurs during oil extraction by solvents, producing aldehides, cetones and n-hexanal, that cause undesirable flavors and lack of stability of the oil during storage (Rackis et al., 1979). These enzymes can be inactivated by heat, but this method is expensive for the seed processing industry. Besides, the high temperatures desnaturate and insolubilize the principal proteins of the seed reducing the nutritional value of the residual cake.

One strategy to improve oil quality without changing it, is to eliminate the lipoxygenase enzymes. Axelrod et al. (1981) describes three forms of the enzymes: L_1, L_2, and L_3 that can be recognized by electrophoresis in polyacrylamide gels. This analysis allowed the identification of soybean mutant lines with null alleles for each form of the enzymes: l_{x1}, l_{x2}, and l_{x3} respectively, and some lines with combinations of these alleles that are useful for breeding programs too. However, the double recessive genotype $l_{x1}l_{x1}l_{x2}l_{x2}$ have not been obtained so far by recombination due to tight linkage in repulsion between both loci. The double recessive finding would advance breeding progress.

The objective of this work was to find individuals with l_{x1} l_{x1} l_{x2} l_{x2} genotype and to determine the frequency of recombination between of loci L_1 and L_2.

Material and Methods

Plant material

Artificial crosses were made at Balcarce Exp. Sta. (Province of Buenos Aires, Argentina) in 1994, between the lines PI 86023 (L_2 -less, genotype $L_{x1}L_{x1}l_{x2}l_{x2}L_{x3}L_{x3}$) and an L_1-L_3- less line provided by the CNPSo of Londrina, Brazil. This L_1-L_3- line genealogy is:(Suzuyutaka^6 x PI 408251) x (Suzuyutaka^6 x Wasenatsu). 11 F_1 seeds were obtained from this cross, with genotype $L_{x1}l_{x1}L_{x2}l_{x2}L_{x3}l_{x3}$. 5 out 11 seeds were harvested and planted in Nebraska for generation advance. F_2 seeds were harvested in October of 1994. 707 F_2 seeds were analyzed by electrophoresis and the double recessive was not found. So, F_2 seeds with genotype L_{x1}-$l_{x2}l_{x2}$- $yl_{x1}l_{x1}L_{x2}$- were selected and planted in the field in October of 1995. 117 individual F_2 plants were harvested in 1996 for $F_{2:3}$ lines derivation. In each $F_{2:3}$ line, 10 individuals were analyzed by electrophoresis (1286 individuals) in order to safe (P=0,94) the identification of recombinant genotypes.

Electrophoresis

The technique of vertical electrophoresis with small gels of polyacrylamide was used (SDS-MINI-PAGE) in order to identify the bands corresponding to the enzymes. Seeds of lines PI 86023 and non L_1 and L_3 and the commercial cultivar Carmen INTA, were used as testers. The method used by Salines et al. (1997) was performed for protein extraction.

Statistic analysis

The statistic program TRP (Mansur et al., 1990) was used to calculate the necessary population size to recover 1 individual with genotype $l_{x1}l_{x1}l_{x2}l_{x2}$ and the minimum necessary probability to safe the identification of recombinant individuals. The population size analyzed allowed to formulate a test of hypothesis (α= 0,06) for a percentage of recombination of 6% between both loci.

Results and Discussion

The results of electrophoretic analysis are shown in Table 1.

The double recessive genotype ($l_{x1}l_{x1}l_{x2}l_{x2}$) was not found in the $F_{2:3}$ lines, therefore, the hypothesis that the percentage of recombination between both loci is 6% or more, was rejected.

In order to find double recessive individuals it is necessary to study a segregating population with more number of individuals.

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Table 1. Number of individuals analyzed by electrophoresis and corresponding genotypes.

Number of individuals	Genotype
115	L_{x1}-L_{x2}-L_{x3}
71	L_{x1}-L_{x2}-$l_{x3}l_{x3}$
347	L_{x1}-$l_{x2}l_{x2}L_{x3}$
213	L_{x1}-$l_{x2}l_{x2}l_{x3}l_{x3}$
272	$l_{x1}l_{x1}L_{x2}$-L_{x3}
268	$l_{x1}l_{x1}L_{x2}$-$l_{x3}l_{3}$
-	$l_{x1}l_{x1}l_{x2}l_{x2}L_{x3}$-
-	$l_{x1}l_{x1}l_{x2}l_{x2}l_{x3}l_{x3}$
1286	

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Use of Pod Width as Visual Score in Substitution of One Hundred Seed Weight on Vegetable Soybean

Introduction

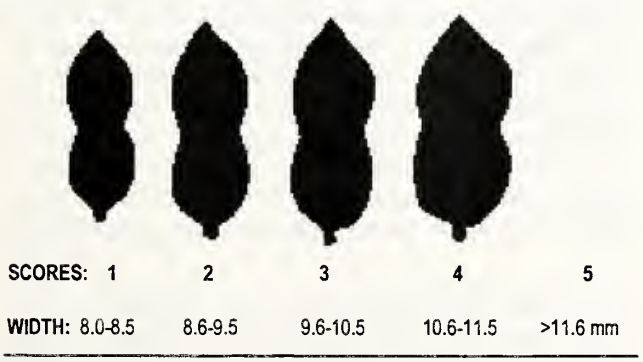
Soybean is a plant with ability to substitute animal proteins. There is one specific soybean for direct human consume called vegetable soybean or edamame. In this group is essential that one hundred seed weight (HSW) equals 20 grams or more, therefore in diverse breeding programs with objectives of creating a vegetable soybean cultivar, there are grain size evaluations like the measurement of pods by pachymeter or weighting one hundred seeds samples, the process is indubitably laborious and demands much time for data acquiring, all breeders know how difficult it is to obtain the grain size by usual widespread methods. Then it is proposed in this work the utilization of pod width as visual score (P W V) as a parameter to facilitate the selection in substitution to the process before mentioned.

Material and Methods

72 crosses were evaluated in field test. In this experiment it was adopted the complete randomized blocks design with 6 replicates and 12 plants per replication. For field evaluation for others characters during harvest or just before (at maximum grain filling stage (R_6 for FEHR & CAVINESS, 1979), it is attributed a note (PWV) to the pod situated in central portion of stem and it represents the general average of all the other pods.

The method is based on the use of the scale showed bellow:

Figure 1. Scale of Visual Score for Pod Width



Pearson's phenotypic correlation was estimated for HSW by PWV and genetic diversity estimation or genotypic determination coefficient (R^2) for mean topcrosses of P W V and H S W was employed due to the fixed effect for treatments, this estimation substitutes the broad sense heritability.

Results and Discussion

If necessary the number of evaluated pods could be increased up to four or more per plant and then use an average number. The PWV method is effective when there is no soil hydric deficit during grain filling period, it could interfere in grain formation and the pod will be coreless.

The knowledge of the nature and the magnitude of the relation between P W V and HSW is important for recommendation of this method as visual score, looking forward to estimate the influence of each other (VENCOVSKY & BARRIGA, 1992; JOHNSON et al., 1985) and to confirm the results from FRANK & FEHR (1981) and BRAVO et al. (1980) where pod width was used for indirect selection of grain weight showed better performance compared to grain weight perse.

Results that support the use of P W V in substitution to HSW are shown bellow.

Due to the fixed effect of genotypes in the experiment the broad sense heritability is called genetic diversity or genotypic determination coefficient, but every estimation is similar, only the denomination has changed.

Table 1. Estimation of genetic diversity or genotypic determination coefficient (R^2) for mean topcrosses of P W V and H S W.

Character	R^2%
H S W	96.07
P W V	90.95

Both values obtained in Table 1 were high, for H S W the results support the observations of diverse authors and more recently of KONIECZNY et al. (1994) and MAURO et al. (1995). The P W V showed a value close to the one observed by BRAVO et al. (1980), this result indicates that both H S W and P W V present high heritability, receiving few environmental influence and thus it is possible to select both characters with more reliability.

Table 2. Estimation of Pearson's phenotypic correlation between H S W and P W V for topcrosses between vegetable soybean and Doko or FT-2 cultivars.

Genotypes	Pearson's correlation %
all 72 topcrosses	94
40 Doko's topcrosses	94
32 FT-2's topcrosses	94

The phenotypic correlation between H S W and P W V shown in table 2 for all 72 topcrosses, and divided into 40 Doko's topcrosses and 32 FT-2's topcrosses, coincidently displayed equal (94%) and high values for the three groups, and higher than obtained by FRANK & FEHR (1981); SHANMUGASUNDARAM & CHUNG-RUEY (1982) and FRASER et al. (1982) and SAKA et al. (1996). This indicates that the use of size of one pod representing the others leads to identical selection efficiency in relation to the grain size.

Conclusion

Based on the results shown in both tables, the use of P W V is viable in substitution to the most usual methods for grain size evaluation.

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Genetics of Leaf Waviness in Soybean

Introduction

Leaf waviness of the margin of soybean leaflets is controlled by two recessive alleles, lw1 and lw2 (Rode and Bernard, 1975). They observed different levels of waviness in different varieties. There is a degree of waviness (unpublished results) in plants of the 3t (Fg1 Fg3) flavonol class (Buttery and Buzzell, 1976, 1987). An attempt to develop lw1lw2 isolines differing in Fg1/fg1 and Fg3/fg3 was unsuccessful which indicated that flavonol glycosides affect waviness. Rode and Bernard (1975) observed that wavy leaf is not expressed in the presence of T which results in quercetin and kaempferol glycosides in soybean leaves versus only kaempferol glycosides in the presence of t (Buttery and Buzzell, 1973). Using segregating populations we studied the effect of T, Fg1, Fg3 and the presence/absence (Wm/wm) of flavonols on the waviness of leaf margins.

Materials and Methods

Flavonol classes were identified by 2-way thin layer chromatography following Buttery and Buzzell (1973) and genotypes assigned according to Buzzell and Buttery (1973, 1974). We made three crosses with the wavy leaf Harosoy lw1lw2 isoline L65-461:

X794 L65-461 (t lw1lw2 fg1fg3) x X523A (T Lw1lw2 fg1fg3)	[6t x 6T]	[wavy x non-wavy]
X796 L73-67 (t Lw1lw2 Fg1fg3) x L65-461	[2t x 6t]	[non-wavy x wavy]
X795 OX731 (t Lw1 Lw2 fg1Fg3) x L65-461	[4t x 6t]	[non-wavy x wavy]

To further test the affect of flavonol glycosides we made a cross of the wavy leaf Harosoy isoline (L65-461) with a Harosoy isoline (OX281) of the wmwm genotype which does not have flavonol glycosides in the leaves (Buzzell *et al.*, 1977). OX281 is also w1w1; with w1 and wm being closely linked (Buzzell *et al.*, 1977), white flowers (w1) serve as a marker for wm.

Results

The flavonol genotypes for some of the parent lines reported by Rode and Bernard (1975) are as follows:

	Leaf		Flavonol	
Strain	Margin	Genotype	Class	Genotype
Clark	Non-wavy	Lw1Lw1 lw2lw2	6T	TT fg1fg1 Fg2Fg2 fg3fg3 Fg4Fg4
Harosoy	Non-wavy	Lw1Lw1 lw2lw2	6t	tt fg1fg1 Fg2Fg2 fg3fg3 Fg4Fg4
T176	wavy	lw1lw1 lw2lw2	6t	tt fg1fg1 Fg2Fg2 fg3fg3 Fg4Fg4
T205	wavy	lw1lw1 lw2lw2	6t	tt fg1fg1 Fg2Fg2 fg3fg3 Fg4Fg4
T117	wavy	lw1lw1 Lw2Lw2	4t	tt fg1fg1 Fg2Fg2 Fg3Fg3 Fg4Fg4

The three crosses gave 3:1 segregations in the F_2 for 6T and 6t, 2t and 6t, and 4t and 6t, respectively (Table 1). The F_2 segregations fit a 15:1 ratio for two genes affecting leaf waviness (Table 2). The $F_{2:3}$ segregations indicated that T, Fg1 and Fg3 are each epistatic to lw1lw2 (Table 2). Since Fg3/fg3 was segregating in the crosses used by Rode and Bernard (1975) to identify Lw2/lw2, Fg3/fg3 may be the same gene as Lw2/lw2. Additional proof of this is a non-wavy Clark isoline, L79-1685, developed as a tt lw1lw1 Lw2Lw2 genotype using T117 as a source of Lw2 (Bernard et. al., 1991). It has a flavonol phenotype of 4t indicating that it has Fg3 instead of the Clark fg3fg3 genotype.

The F_2 population of OX281 x L65-461 was classified has having non-white (purple or magenta) and white flowers, and having normal and wavy leaves (Table 3). All plants with wavy leaves had flowers that tested positive for the presence of flavonols using ammonia fuming as done by Buzzell *et al.* (1977), thereby indicating that Wm was present in those plants. The only white flowered plants with wavy leaves were the two w1Wm recombinants.

Flavonol glycosides are involved in the expression of normal/wavy margins of soybean leaflets. Kaempferol glycosides involving Fg1 and Fg3 are epistatic to the expression of lw1 waviness. It is interesting that Fg1 and Fg3 together results in some waviness of leaf margins in the presence of Lw1, whereas, separately Fg1 and Fg3 each block the expression of leaf waviness caused by lw1. We have not observed any effect of Fg2 and Fg4 on leaf waviness; possibly this is because the flavonol glycoside genes differ in function. Fg1 and Fg3 are involved in the addition of glucose whereas Fg2 and Fg4 involve the addition of rhamnose (Buttery and Buzzell, 1975). T is also epistatic to the expression of lw1 waviness; this may simply be caused by the production of quercetin glycosides resulting in much less kaempferol glycosides in the leaves (Buttery and Buzzell, 1973).

Conclusions

Some of the flavonol glycosides affect the expression of leaf-margin waviness. Fg3/fg3 and Lw2/lw2 may be the same gene.

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Table 1. F_2 segregation for flavonol classes in three crosses

		X794		X796		X795	
	Expected		Observed		Observed		Observed
Ratio	No.	Classes	No.	Classes	No.	Classes	No.
3	37.5	6T (T_)	39	2t (Fg1_)	40	4t (Fg3_)	38
1	12.5	6t (tt)	11	6t (fg1fg1)	10	6t (fg3fg3)	12
	50.0		50		50		50
Chi-square			0.240		0.667		0.027
P			0.7-0.6		0.5-0.4		0.9-0.8

Table 2. F_2 and $F_{2:3}$ segregation for wavy leaf

		X794 Lw1lw1 Tt	X796 Lw1lw1 Fg1fg1	X795 Lw1lw1 Fg3fg3
	Expected No.	Observed No.	Observed No.	Observed No.
F_2 Segregations				
Normal (15)	46.9	48	48	46
Wavy (1)	3.1	2	2	4
Chi-square		0.065	0.065	0.278
P		0.8-0.7	0.8-0.7	0.6-0.5
$F_{2:3}$ Segregations				
Normal	21.9	19	23	17
Seg 15:1	12.5	12	11	18
Seg 3:1	12.5	17	12	11
Wavy	3.1	2	2	4
Total	50.0	50	50	50
Chi-square		2.414	0.645	3.957
P		0.5–0.3	0.9–0.8	0.3–0.2

Table 3. F_2 segregation in a cross of non-wavy, white flowered OX281 (w1w1wmwmLw1Lw1 lw2lw2) x wavy leaf, coloured flower L65-461 (W1W1WmWmlw1lw1lw2lw2)*

	Purple & magenta flowers (W1_Wm_/wmwm)		White flowers (w1w1) (wmwm)	(Wm_)**
	Normal leaves (Lw1_)	Wavy leaves (lw1lw1)	Normal leaves (Lw1_)	Wavy leaves (lw1 lw1)
F_2 plants	999	213	423	2

*OX281 and L65-461 are Harosoy isolines
**Based on ammonia fuming of the flowers

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Inheritance of Autoregulation Mutants in Elgin 87 Soybean Resulting in Supernodulation Phenotype

Introduction

Mutagenesis of Elgin 87 soybean was carried out with the objective of obtaining mutants affecting the autoregulation of nodulation by *Bradyrhizobium japonicum* as given in the preliminary report by Buzzell *et al.* 1990.

Materials and Methods

Elgin 87 seeds were treated with ethylmethane sulfonate at 0.04M for 12h after soaking for 12h in aerated distilled water following the procedures of Wilcox *et al.* 1984. Seeds were planted in peat pots and subsequently transplanted to the field at the Ridgetown College by G. R. Ablett. M_1 plants were harvested individually.

Ten M_2 seeds of each of 999 plants were planted in vermiculite/perlite culture with 14 mM combined N and *Bradyrhizobium* strain USDA 110 at Harrow. Supernodulation mutant plants were identified in five M_2 families after which the remaining seed of each of these families was used to determine the number of mutant plants in each family. The genetically effective cell number was estimated following Li and Redei (1969).

Each mutant was crossed with Elgin and OX717 (derived from Elgin^2 X Elgin 87) for an inheritance study. Mutant line E391 was crossed to the other four mutants for allelism tests. Putative F_1 plants were grown and harvested separately. F_2 and F_3 plants were grown in either vermiculite/perlite culture or soil and rated for nodulation at about the beginning of flowering.

In order to test a two-gene hypothesis for autoregulation, lines were obtained from two $F_{1:2}$ progeny of E391 X OX717 that segregated differently (i.e. 15 normal: 1 supernodulating) from other progenies. Seven lines that were homozygous for normal nodulation were obtained from seven $F_{3:4}$ families segregating 3 normal: 1 supernodulating. Four of the lines were derived from an $F_{2:3}$ family that segregated 24 normal: 2 supernodulation and the other three lines came from an $F_{2:3}$ family that segregated 39 normal: 2 supernodulation. These seven lines were crossed to Elgin, F_1 plants were grown, and F_2 families were screened as described above.

Results and Discussion

Supernodulation mutants were identified in five M_2 families (Table 1). The genetically effective cell number (GECN) at the time of mutation averaged 3.8 but varied from 1 to 8 indicating that the mutations occurred at different times during the treatment process.

The supernodulation mutants were pure-breeding for the supernodulation trait when isolated and grown in subsequent tests (results not shown). Allelism tests indicated that the allele(s) for supernodulation in E391 are allelic with those in E300, E420, E592 and E714 (Table 2), therefore all five mutations are at the same locus. All mutants are nitrate tolerant, having been obtained in the presence of a high level of combined nitrogen. The supernodulating mutants produced more nodules, but grew and yielded less well than the wild type. Grafting experiments showed that supernodulation was shoot controlled. These features were also found in the soybean nodulation mutants produced in Canberra (Gresshoff et al 1988) and Urbana (Cho and Harper 1991).

The F_2 from crossing E300, E420 and E592 to Elgin and OX717 gave a good fit to a 3:1 ratio expected for the segregation of a dominant allele for normal nodulation and a recessive allele for supernodulation (Table 3). However, in the cross of E391 to OX717 one F_2 gave a good fit to a 3:1 ratio and the other two gave a good fit to a 15:1 ratio which would be expected with segregation of two recessive alleles controlling supernodulation. The cross of E714 to Elgin and OX717 did not fit either a 3:1 ratio or a 15:1 ratio; it appeared to be a 3:1 segregation with a deficiency of supernodulating plants in six of the eight F_2 families.

F_3 tests of four F_2 families that had a 3:1 ratio gave a good fit to the expected 1:2:1 ratio of normal nodulation: segregating:supernodulation and the one F_1 family that had a 15:1 F_2 ratio gave a good fit to an expected 7:8:1 ratio (Table 4).

The results in general indicate the presence of a single recessive allele resulting in supernodulation as found by Carroll *et al.* (1988) and by Harper and Nickell (1995) for hypernodulation (rj7). However, there were two F_2 segregations involving E391, one of which was confirmed in the F_3, that indicated the presence of a second gene with similar function in autoregulation. It was hypothesized that there are two loci, each with dominant alleles, involved in autoregulation of nodulation in soybean (Buzzell *et al.* 1990). A test of this hypothesis that recessive alleles at two loci are needed for supernodulation to be expressed did not identify two loci in that there was no segregation in seven testcrosses (results not shown). An alternative hypothesis is that another mutation occurred subsequent to the occurrence of the supernodulating mutation in E391; if this mutation had a homozygous lethal effect in the embryo it could have caused a disturbance in the supernodulation segregation.

From backcrossing the E420 mutant to OX717, pure-breeding supernodulating lines E420BC1, E420BC2 and E420BC3 have been selected. Genetic type number T_____ has been assigned to the E420BC3 line.

Conclusions

A recessive mutation affecting the autoregulation of nodulation was obtained by mutagenesis in Elgin 87 soybean. When obtaining a mutant by mutagenesis, mutations that occur during the mutagenesis process but are not being screened for, may affect the segregation ratios of the selected mutant.

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Table 1. Genetically effective cell number (GECN) and number of supernodulation mutant plants in each of five M_2 families obtained from treating Elgin 87 soybean with ethylmethane sulfonate.

	No. of M_2 plants		
M_1 plant from Elgin 87	Normal	Supernod	GECN
E300	73	6	3.3
E391	8	1	2.2
E420	94	25	1.2
E592	179	6	7.7
E714	188	11	4.5
Mean			3.8

GECN = t/4a; t=total number of M_2 plants in a family; a=number of mutant plants in the M_2 family

Table 2. F_2 tests of supernodulation mutants crossed with E391 (♂) to determine whether the mutations either are at different loci or allelic to one locus.

		No. of F_2 plants	
Mutant line (♀)	No. of F_1	Normal	Supernod
E300	13	0	414
E420	9	0	296
E592	18	0	550
E714	5	0	299

Table 3. F_2 segregation of normal and supernodulating plants in crosses of supernodulation mutants to normal Elgin and OX717 (♂).

	No. of plants			
		F_2		
Mutant line (♀)	F_1	Normal	Supernod	P
		3:1		
E300	18 (0)*	943	309	0.8-0.7
E391	1A**	280	86	0.6-0.5
E420	29(1)	1542	524	0.8-0.7
E592	35(2)	1717	525	0.10-0.05
E714	16(2)	1457	416	0.005-0.001
		15:1		
E391	1B**	68	6	0.6-0.5
	1C**	108	4	0.3-0.2

*Numbers within parentheses are those F_2 families that did not fit ($P<0.05$) a 3:1 or 15:1 ratio; **F_1 plants 1A, B and C

Table 4. Numbers of homozygous normal, heterozygous, and homozygous super-nodulating F_2 plants identified by F_3 tests of supernodulation mutants crossed to OX717 (♂).

		No. of F_2 plants				
Mutant line (♀)	F_2 ratio	Normal	Seg.	Supernod	Expected $F_{2:3}$ ratio	P
E300	3:1	13	16	10	1:2:1	0.5-0.3
E391	15:1*	27	18	4	7:8:1	0.3-0.2
E420	3:1	13	20	7	1:2:1	0.5-0.3
E592	3:1	12	12	13	1:2:1	0.2-0.1
E714	3:1	7	16	6	1:2:1	0.9-0.8

*From F_1-1B in Table 3

Table 5. F_3 segregation of normal and supernodulating plants from heterozygous F_2 plants identified in the F_3 tests of crosses of supernodulation mutants to OX717 (♂)

		No. of F_3 plants			
Mutant line (♀)	No. of F_2 plants	Normal	Supernod	Expected Ratio	P
E300	16	281	88	3:1	0.6-0.5
E391	11*	343	20	15:1	0.6-0.5
	7**	149	43	3:1	0.4-0.3
E420	20	289	89	3:1	0.6-0.5
E592	12	175	36	3:1	0.5-0.4
E714	16	205	61	3:1	0.01-0.005

*From F_1-1B and F1-1C in Table 3; **From F_1-1A in Table 3

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A Codominant SCAR Marker Linked to SMV Resistance Gene Rsa

Introduction

Soybean Mosaic Virus(SMV) is considered to be one of the most important viral pathogens of the cultivated soybeans and can result in serious yield reduction and seed-quality deterioration. SMV is seedborne or transmitted by aphids in a nonpersistent manner. Therefore, resistance to SMV in soybean cultivars provides the most effective and economical control against this virus.

Host resistance to SMV has been identified in various soybean cultivars and a number of cultivars with a broad spectrum resistance to SMV have been identified. The existence of a single dominant gene and independent resistant locus, allowing resistance to a strain of SMV, was demonstrated by genetic analysis. Breeding resistant varieties to SMV still represents a major effort and long-term investment. Marker-assisted selection will certainly provide a major improvement for gene introgression into susceptible lines and avoid cumbersome pathogenicity tests. The conversion of mapped RFLP and RAPD markers to their sequence-tagged site((STS) or sequence characterized amplified regions(SCAR) counterparts has proven an effective tool of obtaining easy-to-use, reliable markers of important genes in marker assisted selection.

In this paper, we describe the development of a codominant SCAR marker linked to Rsa, a single dominant gene for resistance to soybean mosaic virus strain Sa.

Materials and Methods

Plant materials and linkage analysis

A F2 population with 81 individuals from a cross between a resistant cultivar, Kefeng No.1 and a susceptible cultivar, Nannong 1138-2 was used to map Rsa gene . F1 plants of the cross and F3 lines derived from above F2 population were evaluated for response to SMVstrain Sa in order to verify genotypes of F2 plants. Seven resistant cultivars such as PI486355, Kefeng No.1, Pixianchadou, Fengxianhongguandou, NJR25-8, NJR32-8, Lichengxiaoliqing and 7 susceptilble cultivars such as Nannong 1138-2, Huaidou No.2, Suxie No.1, Suxie 18-6, Zhongdou 24, Nannong 73-935, Davis were adopted to identify genetic diversity in cultivated soybeans. Linkage analysis was performed with the program Mapmaker 2.0, Kosambi function was selected. Linkage was considered significant if the LOD score was greater than 3.0.

DNA extraction, Pool making and Primer screening

Total DNA was extracted from seedling or ripening leaves using the CTAB method with slight modifications. Fifteen resistant F2 individuals and 15 susceptible F2 individuals were selected for pool making. Equal volumes (2g) of DNA from each individual were pooled to obtain two bulks. Nine hundred decamer primers (Operon Technologies, Inc. Alameda, CA) were used to screen the two bulks.

Cloning and Sequencing of RAPD products

RAPD analysis were carried out with 10-mer primers according to the procedure of Operon. The amplified products of linked RAPD markers from the resistant parent Kefeng No. 1 were retrieved from 1.4% agarose gels with micropipettes and were reamplified using the same primer that identified the RAPD polymorphism. The reamplification products were resolved by gel electrophoresis, the band was excised and purified by the 'Gene Clean' kit, and blunt-end ligated into the pGEM-T easy vector from Promega. A portion of the cloned RAPD products were digested with EcoR I and the fragments were identified by both size comparison with the original RAPD marker and Southern blot hybridization with the insertional fragment as a probe. Double-stranded sequencing of the cloned fragment was done by the dideoxy-chain termination method with 373A DNA Sequencer.

Design of SCAR primers and Amplification conditions

Based on the sequence of the RAPD marker OPW-05_{660} from Kefeng No.1, two specific oligonucleotides were designed and then synthesized to be used as SCAR primers. Each primer contained the original 8 or 10 bases of the RAPD primer followed by the 10 adjacent nucleotides(Table 1).

Table 1. Sequences of the two SCAR primers derived from the sequence of OPW-05_{660}

Primers	Sequences 5' 3'
primer 1	CGGATAAGAACACGGGCC
primer 2	GGCGGATAAGCATTGTAGCT

Amplification reaction was carried out in 25l of reaction volume containing 10 mM Tris-HCL, 50mM KCL, 100M dNTPs, 0.1M each primer, 1 unit Taq DNA polymerase, 2.5 mM Mg^{2+} and 30ng template DNA. Reactions were performed in a Perkin-Elmer Cetus DNA-thermocycler 9600. Amplification conditions were 1 cycle of 95, 3 minutes; 35 cycles of 95, 50 seconds, 58, 50 seconds, 72, 1.5 minutes; 1 cycle of 72, 10 minutes. Amplification products were analyzed by electrophoresis in 1.5% agarose gel.

Results and Discussion

Dominant RAPD Markers

Two dominant RAPD markers, OPAS-06_{1800} and OPW-05_{660}, linked to Rsa (a single dominant resistant gene, reported previously) in coupling phase were identified. Southern blotting showed that both of them were low copy DNAs, and were polymorphic between two parents. The order of these loci and genetic linkage distance were OPAS-06_{1800} 24.3cM Rsa 10.2cM OPW-05_{660}. OPW-05_{660} DNA fragment was reamplified, purified,

cloned and sequenced. Sequence analysis showed that the 10 bases at 3'end was the same as decamer primer OPW-05, but 3 bases were lost at 5'end. Therefore, two SCAR primers including the original 8 or 10 bases of the primer OPW-05 followed by the 10 adjacent nucleotides from two ends were synthesized (Table 1) and used to amplify genomic DNA of parents, F1, F2 plants and 14 soybean accessions.

Codominant SCAR Marker

A polymorphic band with the same size (about 660bp) as OPW-05_{660} was amplified from the resistant parent Kefeng No.1, and two polymorphic bands in different molecular size (one is about 1kb and another is about 1.5kb) were obtained from the susceptible parent, Nannong 1138-2, when annealing temperature was set at 55. With the annealing temperature raising degree by degree up to 60, the amplification products were consistent. Therefore 58 was adopted as annealing temperature in the following amplification, and the three fragments mentioned above were amplified from F1 individuals, length polymorphisms obviously existed between the two parents. Accordingly, the SCAR marker was scored as a codominant one and named SCW-05. The segregation of SCW-05 in 81 individuals of F2 population showed that heterozygous and both homozygous genotypes were clearly distinguishable. Cosegregation analysis with genotypes of these F2 plants concerning resistance verified by evaluation against F3 lines indicated that the genetic linkage distance between SCW-05 and Rsa was 7.7cM.

Genetic diversity detected by SCW-05

Genomic DNA from 7 cultivars resistant to Sa and 7 cultivars susceptible to Sa were used as templates for PCR amplification with the two SCAR primers. A band of about 660bp in size was amplified from 7 resistant cultivars: PI486355, Kefeng No.1, Pixianchadou, Fengxianhongguandou, NJR25-8, NJR32-8 and Lichengxiaoliqing, but not from those susceptible cultivars. On the other hand, two bands of about 1kb and 1.5kb were simultaneously amplified only from 7 susceptible cultivars: Nannong 1138-2, Huaidou No.2, Suxie No.1, Suxie 18-6, Zhongdou 24, Nannong 73-935 and Davis. The genetic diversity detected by SCW-05 in these 14 cultivars was consistent with their resistance or susceptibility. Southern blotting showed that the three fragments were homologous, the difference in size between them is probably caused by deletion, insertion or internal sequence sites.

Compared with RAPD and RFLP markers, the codominant SCAR marker has several advantages. It is easy to use and more reproducible, requires small sample and has high throughput, does not need the use of radioactive isotopes. The codominance of it increases the amount of information in each F2 individual. It can be applied to early selection in disease resistance breeding programs or identification and fingerprinting analysis of resistant cultivars. Mapping of SCW-05 and Rsa on linkage groups will facilitate the simultaneous improvements of SMV-resistance and other agronomically important traits in soybean.

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A Preliminary Study on Mutation Site of a New Electrophoretic Variant of Sbti-A_2 from Soybean Seed Storage Protein

Introduction

The Kunitz trypsin inhibitor was isolated and crystallized by Kunitz in 1945 from soybean seed protein. It was designated SBTi-A_2 through electrophoreses and genetics study. There were three alleles, Ti^a, Ti^b, Ti^c, in this locus. In 1992, Zhao Shuwen reported that a new variant of SBTi-A_2, Ti^d, was found from soybean seed in Gansu province of China. The Ti^d protein was purified in our lab. Compared to Ti^a, the mutation position was identified to be located on a 9.8 kD fragment, which corresponds to 1 ~ 84 amino acids of the protein.

Materials and Methods

Ti^d and Ti^a were reduced and S-carboxymethylated by the method of Seung-Ho KIM, et al (1985). The alkylated proteins were reacted with a 400-fold molar excess of CNBr over the methionine residues in 70% formic acid for 2 days at room temperature. The CNBr-fragments were analyzed by PAGE and Tricine/SDS-PAGE.

Results

1. The results of 16.5% SDS-PAGE showed that the Met residues in Ti^d were the same as those in Ti^a (Fig. 1).

2. There were three distinct bands in 12% PAGE of the CNBr-fragments of Ti^d and Ti^a (Fig.2), d_1, d_2, d_3 and a_1, a_2, a_3. The mobility of d_3 and a_3 were different, while d_1 and a_1, d_2 and a_2 were the same, indicating the mutation was taken on fragment a_3 or d_3. 16.5% SDS-PAGE of d_1, d_2, d_3, a_1, a_2, a_3 showed that a_3 or d_3 was the 9.8 kD fragment of the proteins (Fig.3). As a result, the mutation position was identified to be located on a 9.8 kD fragment, which corresponds to 1 ~ 84 amino acids according to the amino acids sequence of Ti^a.

Discussion

The mobility of d_1 and a_1, d_2 and a_2 were the same but the mobility of d_3 was slower than a_3. It was consistent with the result that Ti^d migrates more slowly than Ti^a. The mutation was taken on the fragment of 1 ~ 84 amino acids in which the reactive site was located. It was consistent with the result that the K_1 value of Ti^d was a little higher than that of Ti^a (1996).

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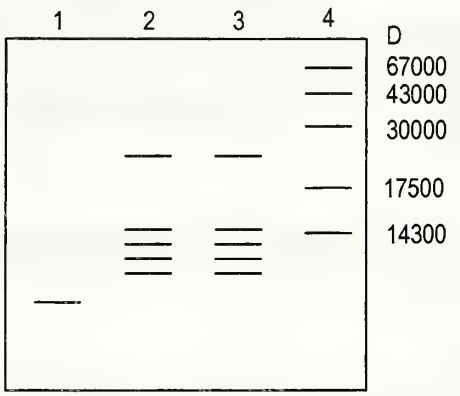

Figure 1. Analysis of CNBr-hydrolysis-derived fragments of alkylated Ti^d and Ti^a on SDS-PAGE
1. Insulin(5600)
2. CNBr-hydrolysis-derived fragments of alkylated Ti^d
3. CNBr-hydrolysis-derived fragments of alkylated Ti^a
4. MW markers

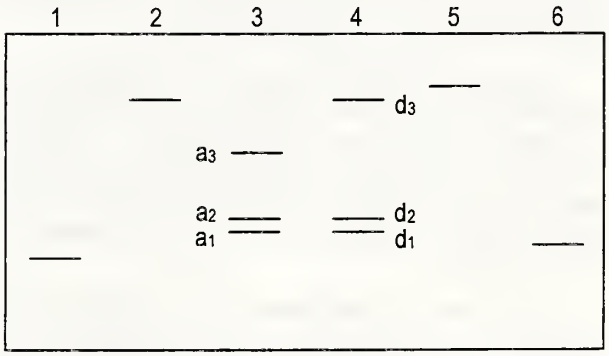

Figure 2. Analysis of CNBr-hydrolysis-derived fragments of alkylated Ti^d and Ti^a on 12% PAGE
1. Ti^a
2. Alkylated Ti^a
3. CNBr-hydrolysis-derived fragments of alkylated Ti^a
4. CNBr-hydrolysis-derived fragments of alkylated Ti^d
5. Alkylated Ti^d
6. Ti^d

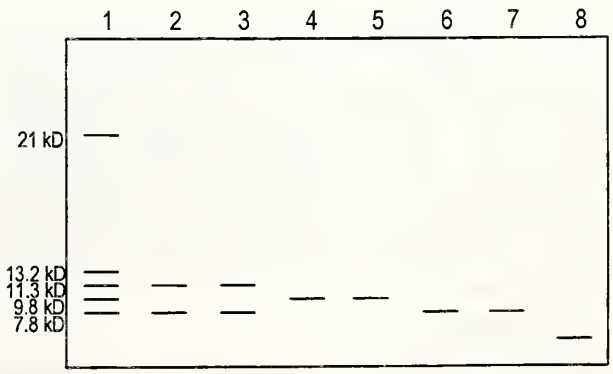

Figure 3. Analysis of CNBr-hydrolysis-derived fragments on 16.5% SDS-PAGE
1. CNBr-hydrolysis-derived fragments of alkylated Ti^a;
2. a_1; 3. d_1; 4. a_3; 5. d_3; 6. a_2; 7. d_2; 8. Insulin (5600).

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A RAPD Marker Linked to the Salt Tolerant Gene in Soybean

Introduction

In China, more than 10,000 soybean germplasm accessions have been identified for salt tolerance, and some accessions with salt tolerance have been found (Shao et al, 1986). Genetic study has been conducted using the crosses of screened salt tolerant accession and salt sensitive accession (Shao et al, 1994). The result shows that salt tolerance is heritable, and controlled by a single dominant gene (Shao et al, 1994). It is coincidence with the previous study finished in US (Abel, 1969).

The salt tolerant screening can only be conducted in the certain regions with irrigation of salt water, but the treatment of salt water is affected by the environments. Compared to traditional field screening, molecular markers are quick and accurate for identifying salt tolerance, and are less likely to have been affected by environments when direct salt treatment is applied in the field.

The purpose of this experiment is to identify RAPD markers tightly linked to the salt tolerant gene by the population, which had been used for genetic study of salt tolerance (Shao et al, 1994). Thus it will be possible to conduct marker assisted selection in soybean breeding program and germplasm identification.

1. Materials and Methods

1.1 Genetic materials

A cross was made between salt tolerant variety (Wenfeng 7) and sensitive variety (Union). Two parents, four F1 plants, and 200 F2 individuals were planted in 1997. They were identified for their salt tolerance in field after irrigating with 4.8ds/m sea water at seedling stage. The phenotypes were scored individually for their salt tolerance and salt sensitiveness. In addition, ten salt tolerant varieties and 12 salt sensitive varieties previously identified were planted and treated with 4.8ds/m salt water in seedling stage.

1.2 DNA extraction

DNA was extracted from two parents, F1, and F2 individuals using CTAB method. The DNA of 20 salt tolerant individuals were mixed in a equal capacity to obtain the salt tolerant pool (1T), and the DNA of 20 salt sensitive individuals were mixed in a equal capacity to obtain the salt sensitive pool (1S). Meanwhile, DNA of both 10 salt tolerant varieties (Tm) and 12 salt sensitive varieties(Sm) were extracted and mixed respectively in the same method.

1.3 PCR reaction

Random 10bp primers for RAPD markers were obtained from Operon. PCR reaction were conducted using 2.5ul 10X PCR buffer, 2.5ul 15mM $MgCl_2$ 1.25ul 2mM dNTP, 1U Taq polymerase and 40ng DNA template in a total volume of 25ul. Amplification were performed in a Hybaid Thermal Cycling System using the following program: after 3min of 94°C, 42cycles of 94°C 15s, 36°C 30s and 72°C 1min following 7 min at 72°C. Amplified products were separated by electrophoresis through 1.3 % agarose gel containing ethidium bromide.

1.4 Screening strategy

Six DNA samples were used for this preliminary study. The RAPD markers were screened using two sources of DNA bulks. One source of DNA pools came from F2 population (including 1T, and 1S), and the other source of DNA pools were mixes of varieties (including Tm and Sm). In addition, two parents of the F2 population were used.

2. Results and Discussion

One of 153 primers was screened out, which might link to the salt tolerant gene because the regular patterns showed among six DNA samples. Both DNA pools of salt tolerance and sensitiveness have PCR products, but show different fragment in size. Three of DNA samples with salt tolerance (including 1T, WenFeng7, and Tm) appeared the same special banding pattern except 1T, which also showed the special pattern for salt sensitiveness. Compared to salt tolerant samples, the other three DNA samples with salt sensitiveness (including 1S, Union, and Sm) had same special smaller size of fragment. This result suggested that this marker might closely related to the salt tolerance or salt sensitiveness and it is codominant.

Observing the electrophoretic pattern of the F1 individual of the cross of Wenfeng 7 and Union, the distinction was obvious. Each of F1 individuals had the bands of both salt tolerance and sensitive parents. This result confirmed that this marker is codominant, which can be used to identify true or false hybrid.

The total of 43 individuals with salt sensitiveness, including 20 individuals used in the pool (1S), were analyzed by this marker. None recombinant has been found. Therefore, we postulate that this marker might be tightly linked to the salt tolerant gene, or might be within the gene.

For the bulk (1T) of 20 DNA samples composed with salt tolerance in the F2 population, some of them showed the special band for salt tolerance, the others had both special bands for salt tolerance and sensitiveness. It is suggested that this marker can be used to identify heterozygous and homozygous of salt tolerance.

This preliminary study imply that this marker might be useful not only for identifying the salt tolerance or salt sensitiveness of soybean germplasm, but also useful for distinguishing the genotypes of salt tolerant individuals, even identifying salt tolerant and salt sensitive in the F1 generation.

By using this marker, we are identifying the remnants of F2 individuals with salt tolerance in the population of cross of Wenfeng 7 and Union. Except traditional genetic analysis of this marker, we will verify this marker by the other two populations of crossing salt tolerance with salt sensitiveness.

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A Study on RAPD Markers of Genes Resistant vs. Susceptible to SCN Race 1 in Soybean

Introduction

The predominant race of the soybean cyst nematode (*Hoterodera glycines* Ichinohe, SCN) in Shandong province, China, was Race 1 (Xing Han, et al., 1997). It was reported that the inheritance of resistance to SCN Race 1 was controlled by three pairs of recessive genes (Caldwell et al., 1960). Recently, there appeared increasingly reports on soybean resistance to SCN, especially on mapping molecular makers linked to resistant genes, but most of them were about resistance to Race 3 (Vierling et al., 1996, Concibido et at., 1996). The present report deals with the RAPD markers of resistant vs. susceptible genes to SCN Race 1 in soybeans by using a BC_1F_2 population.

Materials and Methods

The BC_1F_1 population was derived from (RN-9)[2] X 7605 in which RN-9 was resistant and 7605 was susceptible parent. Five BC_1F_1 derived lines in BC_1F_2 were used in this study. The total number of plants used for RAPD analysis was 64 plants selected from 368 plants of five BC_1F_2 lines. Plants having index of parasitism (IP) of 10 or more were considered susceptible while those having an IP of less than 10 were considered resistant.

Total genomic DNAs were extracted according to the CTAB method. Equal DNAs from all twelve resistant plant s of the five BC_1F_2 lines were mixed to make a resistant bulk, and equal DNAs from susceptible plants of a same line were mixed to make five susceptible bulks corresponding to the five BC_1F_2 lines. Three hundred and twenty primers (Operon Technologies, inc. Alameda, CA) (Table 1) were used to screen between the five susceptible bulks and the one resistant bulk. The primers which produced polymorphic bands were used to amplify genomic DNAs from the parents (RN-9, 7605) and each of the 64 BC_1F_2 plants.

Results and Discussion

The results of the X^2 tests (Table 2) showed that the segregations of resistant to susceptible in each line was fitted well the expected ratio (3:1 in line 10, 15: 1 in line 1 and 98, 63:1 in line 11 and 43). This was in accordance with previous reports that the inheritance of resistance to SCN Race 1 was controlled by three pairs of recessive genes (Caldwell et al. 1960).

Of the three hundred and twenty primers, OPAO19 was found to produce a 1.2 kb polymorphic band which was designated as $OPAO19_{1200}$. $OPAO19_{1200}$ could be produced in 7605, all susceptible plants of BC_1F_2 line 11 (R: S= 1: 63) and 11 of 12 susceptible plants of BC_1F_2 line 43(R: S=1:63), while it could not be produced in RN-9, all resistant plants and other susceptible plants of BC_1F_2 population. Based on the result of genetic experiment, line 11 and 43 had one or two more susceptible genes than other lines. $OPAO19_{1200}$ could only be produced in these two lines. So line 11 and 43 might have one susceptible gene which did not exist in other lines, and $OPAO19_{1200}$ could be related to that gene (Table 3).

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Table 1. Primers used to screen between susceptible bulks of different lines and resistant bulk.

OPB 01-20	OPI 01-20	OPP 01-20	OPV 01-20
OPC 01-20	OPL 01-20	OPR 01-20	OPZ 01-20
OPD 01-20	OPN 01-20	OPS 01-20	OPAW 01-20
OPF 01-20	OPO 01-20	OPT 01-20	OPAO 01-20

Table 2. X^2 tests for each BC_1F_1 derived line in BC_1F_2

Code of BC_1F_2 line	No. of susceptible plant	No. of resistant plant	Ratio tested	x^2	P
1	67	6	15:1	0.4831	>0.250
10	47	21	3:1	1.2549	>0.250
11	86	2	15:1	2.3758	>0.100
			63:1	0.2797	>0.500
43	64	2	15:1	1.1677	>0.250
			63:1	0.9245	>0.250
98	58	5	15:1	0.2639	>0.500

Table 3. $OPAO19_{1200}$ reaction and susceptible genes postulated in BC_1F_2 lines

Code of BC_1F_2 line	Ratio tested	$OPAO19_{1200}$ Reactions*	Postulated susceptible genes
1	15:1	10(-) + 3R(-)	R_1, R_2
10	3:1	7S(-) + 5R(-)	R_1
11	63:1	12S(+) + 1R(-)	R_1, R_2, R_3
43	63:1	11S(+) + 1S(-) + 1R(-)	R_1, R_2, R_3
98	15:1	11S(-) + 2R(-)	R_1, R_2

* The digits are numbers of plants. S and R represent susceptible and resistant, respectively. - and + represent without and with $OPAO19_{1200}$ band, respectively.

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A Virescent Mutant Controlled by a Recessive Nuclear Gene

Introduction

Virescent foliage, a specific chlorophyll deficiency trait, is a useful trait for genetic, developmental and physiological studies, and also it is desirable as a genetic marker. More than 20 genes controlling chlorophyll deficiency were identified in soybeans but no gene locus for typical virescent leaves was reported. Two mutants, T275 and NJ89-3, with virescent characteristics were found but were cytoplasmic-inherited. In 1994, a plant performed with virescent leaves was found from a line of Tongzhoudou at Jiangpu Agricultural Station of Nanjing Agricultural University. The derived lines from the plant showed the same kind of virescent leaves in 1995 and 1996. They performed morphologically very similar to the original line of Tongzhoudou except the young leaf color. It was inferred that the virescent lines, designated as NG96v-1, might be a mutant from Tongzhoudou. The seedlings of NG96v-1 had green cotyledon, but each of the unifoliolate and trifoliolates appeared brightly yellow before it was completely developed, then it turned normal green as the original line. The leaf virescence of NG96v-1 looks like that of T275 and NJ89-3. The present paper deals with the inheritance of the virescent leaves of NG96v-1 to see whether it is the same as the previously reported ones.

Materials and methods

The virescent mutant NG96v-1 was crossed with 4 normal parents, Youchu 4, Xiangshuidou, Dafangfengdingdou and Zigongliuyuehuang, and the reciprocal crosses were made with the parents except the last one (Table 1). A part of hybrid seeds of Cross 1, NG96v-1 Youchu 4, were sent to Hainan island to get F2 seeds during the winter of 1996. Seeds of P1P2F1F2 in cross 1 and seeds of P1P2F1 in Cross 2 through Cross 7 were planted at Jiangpu Agricultural Station of Nanjing Agricultural University on June 11, 1997. After harvest, 30 random F2 plants of Cross 1 and two F1 plants of Cross 5 were taken, and planted as F2:3 lines and F2 plants, respectively, in pots in the autumn, 1997. The leaf color was observed and recorded on per plant base at V2-V3 stage. The 2 criterion was used to test the segregating ratio.

Results and Discussion

The leaf color of all the F1s of seven crosses appeared green as the normal parents, no matter the crosses and their reciprocal crosses. It indicated that no cytoplasmic effect was involved, and some recessive genes might exist for the virescent trait. Segregation of the observed two crosses in F2 occurred. Table 2 showed the proportion of normal to virescent, which fitted a 3:1 ratio. Among 30 F2:3 lines derived from normal F2 plant, the proportion of non-segregating lines to segregating lines was 8:22 which fitted a 1:2 ratio (2=0.34, 2=0.56). The proportion of plant number of the segregating F2:3 lines also fitted the 3:1 ratio. Therefore the results supported the hypothesis of a recessive gene controlling the virescent leaf of NG96v-1.

The co-segregating data of leaf virescence with flower color, pubescence color and leaf shape were obtained from Cross 1. Table 3 showed that the leaf virescence was independently inherited with the other three traits.

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Table 1. The leaf color of the F1s.

Code	Female	Male	Leaf color of F1
Cross 1	NG96v-1	Youchu 4	Green
Cross 2	Youchu 4	NG96v-1	Green
Cross 3	NG96v-1	Dafangfengdingdou	Green
Cross 4	Dafangfengdingdou	NG96v-1	Green
Cross 5	NG96v-1	Xiangshuidou	Green
Cross 6	Xiangshuidou	NG96v-1	Green
Cross 7	Zigongliuyuehuang	NG96v-1	Green

Table 2. Segregation of the observed two crosses.

Code	Generation	Total	Normal plant	Virescent plant	$\chi^2_{(3:1)}$	P
Cross 1	F2	331	258	73	1.38	0.24
Segregating	F2:3	403	305	98	0.07	0.80
Cross 5	F2	129	100	29	0.31	0.58
Pooled		863	663	200	1.44	0.23
Homogeneity					0.32	0.96

Table 3. Test for linkageship between leaf virescence and other three traits.

Combination type*(Expected ratio)	Total	Segregating ratio	χ^2	P
Lv_W1_:Lv_w1w1:lvlvW1_:lvlvw1w1(9:3:3:1)	331	193:65:54:19	1.57	0.67
Lv_T_:Lv_tt:lvlvT_:lvlvtt(9:3:3:1)	329	194:64:54:17	2.09	0.55
Lv_Ln_:Lv_lnln:lvlvLn_:lvlvlnln(9:3:3:1)	331	195:63:56:17	1.68	0.64

* W-w, T-t, and Ln-ln were alleles for flower color (purple to white), pubescence color (tawny to gray), and leaf shape (broad to narrow), respectively; here Lv-lv are assumed temporary for normal to virescent.

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Development and Cytological Features of the Cytoplasmic-Nuclear Male Sterile Soybean Line NJCMS1A

Introduction

The first cytoplasmic male sterile (CMS) soybean line was reported by Davis (1985) in a U.S. Patent, but no further information released. In China, three cytoplasmic-nuclear male sterile line were reported. Sun Huan (1994) developed a cytoplasmic-nuclear male sterile line derived from a cross between *Glycine soja and Glycine max*. Gai et al (1995) found and reported the cytoplasmic-nuclear male sterility from a cross between two soybean cultivars N8855 and N2899. Zhang and Dai (1997) mentioned another cytoplasmic-nuclear male sterile line from a cross between two soybean cultivars. The present report deals with the development and cytological features of NJCMS1A derived from the previously reported cross, N8855 × N2899.

Materials and Methods

1. Development Process of NJCMS1A

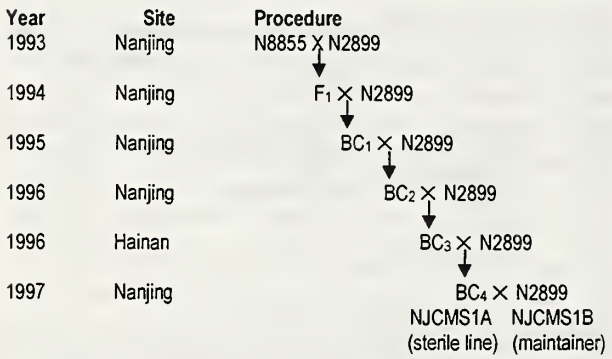

Fig.1. Development process of NJCMS1A and NJCMS1B

Figure 1 showed the development process of NJCMS1A and NJCMS1B, the male sterile line and its maintainer. The cross, N8855 × N2899, was made in 1993. After three times of successive backcrosses, the BC_3 were obtained in Nanjing in 1996. In the winter of 1996, the backcross BC_3 × N2899 was made with 3 hrs additional artificial light in Hainan island, China.

2. Tests of male and female fertility

Pollen germination test was carried out according to Gai (1975). Parallel pollination were made on the male-sterile plants of F_1, BC_1, BC_2, NJCMS1A with N2899 as the pollinator to show whether female fertility was normal with a same success rate.

3. Cytological observation

Individual anthers of NJCMS1A and NJCMS1B were dissected from flower buds of various ages, fixed in 2% glutaraldehyde, dehydrated with acetone, and embedded in Epon8l2 gums. Sections in 40-60nm thickness were obtained by using LKB-V slicer and then stained with $UO_2(CH_3COO)_2 \cdot 2H_2O$ and lead citrate. Specimens were observed and photographed under HITACHI H-600 transmission electron microscope.

General squash preparations and paraffin sections were used to observe cytological features of anther development of both NJCMS1A and NJCMS1B.

Results

1. Development of NJCMS1A

After high male sterility was found in F_1, of the Cross N8855 × N2899 in 1989, this finding was verified five times during 1992-1996 in Nanjing and Hainan Island. The germination ratio of pollen grains were 0-0.32%, while those of pollen grains from F_1, F_2, F_3 plants of the recipraral cross N2899 X N8855 were 76-96.8% (Table 1.). It was inffered that female parent N8855 might have cytoplasmic male sterile gene and the male sterility of F_1 of N8855 × N2899 was due to the interaction between nuclear and cytoplasmic genes. Four cycles of nuclear substitution backcrosses had been conducted since 1994. The percentage of male sterile plants and the degree of male sterility increased with the advance of backcross times (Table 2.). The ratio of male sterile plants in BC_4 was 98.2% and the germination ratio of all male sterile plants were 0%. A cytoplasmic-nuclear male sterile line (designated as NJCMS1A) and its maintainer N2899 (designated as NJCMS1B) were obtained.

2. Features of NJCMS1A

Except the leaves remained to be green and a large number of undeveloped little young pods (0.3-1.2cm in length) set on stem at maturity, NJCMS1A has a same phenotypical performance like its maintainer NJCMS1B in plant height, leave shape, flower color and so on. It is interesting that 2-3 styles in one flower were observed in male sterile plants of BC_1, and BC_2, which could produce 2-3 pods after artificial pollination.

3. Female fertility

The parallel pollination trial showed the female fertility of the male sterile plants of F_1, BC_1, BC_2, NJCMS1A was normal with a same pod set ratio when pollinated with N2899 which was equal to that of N8855 (Table 3.).

4. Cytological features of NJCMS1A

Comparative observation on anther development in NJCMS1A and NJCMS1B showed that from meiosis to late mononucleate stage, anther development of NJCMS1A was not abnormal and that the pollen abortion of NJCMS1A occurred in early dinucleate stage. There appeared more number of small vacuoles in pollen cytoplasm of NJCMS1A than that of its maintainer at the stage of reproductive cell close to nutritive cell nuclei. After reproductive cell was away from nutritive cell nuclei, starch grains could not be

produced in cytoplasm, nuclei stopped developing, reproductive cell further degenerated, the part of cytoplasm disintegrated, and vacuoles expanded. In comparison with all the reported nuclear male sterile mutants, the pollen abortion of NJCMS1A happened relatively late.

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Table 1. Pollen germination ratio of the F_1 of recipraral crosses between N8855 and N2899.

Site	Emergence date	Pollen germination ratio (%)	
		(N8855 × N2899)	(N2899 × N8855)
Hainan	11/21/1994	0-0.13	77-92
Nanjing	05/13/1995	0-0.08	79-87
Nanjing	07/04/1995	0-0.32	76-96.8

Table 2. Percentage of male-sterile plants and pollen germination ratio in backcross generations.

Backcross generation	Percentage of pollen germination (%)	Sterile plants: fertile plants	Percentage of sterile plants (%)
BC_1	0-0.08	127: 138	47.9
BC_2	0-0.02	267: 181	59.5
BC_3	0	150: 19	88.7
BC_4	0	55: 1	98.2

Table 3. Tests for female fertility.

Year	Cross	No. of pollinated flowers	No. of pod set	Success ratio (%)
1995	F_1 × N2899	400	42	10.5
	N8855 × N2899	400	45	11.3
1996	BC_1 (sterile plants × N2899)	250	37	14.8
	BC_2 (sterile plants × N2899)	250	39	15.6
	N8855 × N2899	250	36	14.4
1997	NJCMS1A × N2899	200	9	4.5
	N8855 × N2899	200	10	5

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Evaluation of Methods for Seed Size in Interspecific Crossing of Soybeans

Introduction

Soybean (Glycine max) is one of the world's most important crops, which provides a large amount of protein and fat for the people. For such an important crop, it's breeding still relies on a relatively narrow base of genetic resource (Lohnes and Bernard, 1991), and it restricts the improvement of soybean yield, quality and adversity--resistance. Wild soybean (Glycine soja) germplasms are of great value as potential genetic resources since they possess many valuable genes including those for resistance to diseases and pesticide, and controlling high protein content and yield (Li, 1996). Although there exist so many trait genes among wild soybeans, soybean interspecific hybrids are usually characterized by vining stem, lodging and small seed (Wang, et al., 1986). During the past years, many studies have been performed on genetic analysis of characters of interspecific crossing of soybeans (Williams, 1948; Weber, 1950), and utilization methods of them concentrated on how to select parents for interspecific crossing (Zhang, 1985), and which generation to choose for backcrossing by cultivated soybeans (Gai et al., 1982).

However, very little is known about the selection effectiveness of different methods and which characters exerting high correlated selection responses on others in interspecific hybrid soybean populations.

Up to now, numerous selection methods have been proposed for soybean breeding. Of them, mass selection (MS) and pedigree selection (PS) are two ones, and are extensively utilized. Several comparisons of these two procedures have been made in cultivated x cultivated crossing of soybeans (Reaber et al., 1950; Torrie, 1958; Wang et al, 1964). Seed size is considered to be an important character, which can exert great effects of selection on other characters of hybrid soybean populations.

The objectives of this study are to determine if selection for seed size is equally effective using the pedigree and mass selection methods of breeding, and how much degree of effects of seed size selection on other characters in two types of interspecific crossing of soybeans.

Materials and methods

Segregating populations from 2 crosses involving 3 soybean varieties were utilized in the evaluation of 2 selection procedures for seed size. The crosses were made at Experimental Station of Northeast Agricultural University (NEAU) in Harbin, in 1991. Their pedigrees were as follows:

Cross	Parentage
S17	Dongnong42 x S 1 7
G18	Dongnong42 x G18

Dongnong42 was selected for it's large seed, high yield and other desirable agronomic characteristics S17 was a wild, and G18 was a semi--wild soybean. Both of them were high protein content, higher 100--seed weight and more pods and seeds per plant.

In F2 generation, approximately 500 spaced plants were grown for each cross. After the plants harvested and the characters of them measured, about 170 plants of two seed size groups (large and small) were selected from each F2 population. Therefore, each cross was represented by 340 plants. The four groups studied were as follows:

Group	Cross	100--seed weight
1	S17	> 6g
2	S17	< 5g
3	G18	> 8.5g
4	G18	< 7g

From each 170 plants, five seeds were harvested and went into a mass group. These groups (total 4) were grown for 50 rows in F3, separately, and the procedure made was the same as in F2. As a result, about 170 plants from each group in each cross were saved for comparison with those from the PS in F4.

For the PS, 50 plants ranked by largest and smallest 100--seed weight, respectively, were selected out of 340 plants in each cross. In F3, these 50 plants were grown in spaced progeny rows, and each row was bulkily harvested. After the characters of plants were measured, five selections were made from 50 rows by the seed size. These selections formed PS materials for evaluation in F4 test for comparison with selections by the MS.

In F4, the selections of each group within methods were compared in a randomized block design with five replications. The plot consisted of three 4-m rows with a 0.70-m spacing between rows. Each cross was planted as a separate experiment. Five plants were harvested from each row for character measurement. Field tests were conducted in 1995 at the Experimental Station of NEAU in Harbin.

Characteristics measured were: seed yield: grams per plant; maturity: 95% of pods ripe; lodging: scored at maturity with 1=erect and 5=viny; height: centimeters from ground level to terminal node, measured at maturity; stemdiameter: centimeters of middle part of plant stem, measured at maturity, seed size:

grams of 100 air--dried seeds; seeds per plant: number per plant; pods per plant: number per plant; branch number: number per plant.

Results and discussion

Table 1 showed the comparison of selection for seed size. 1 00-seed weight was relatively larger in cross G18 than in cross S17 due to the difference in types of cross. Highly significant differences in seed size existed among selection groups within methods in both two crosses, informing the stratification selection based on the seed size ranked in F2 generation was adaptable.

In cross S17, the MS materials were significantly larger in seed size than the PS materials, and the MS generally had the higher estimates of genetic variance for seed size. The cases were also true in cross G 1 8. This indicated that PS favored genotypes with smaller seeds, and MS was efficient in maintaining large-seeded genotypes and genetic variability for seed size.

It would be pointed out that the selection intensity for seed size of MS made in the study was about 10%, ten times smaller than that of PS, which was 1%. So in the MS selected materials there could include both variances among progenies and within progenies, which in the PS there mainly existed variance within progenies, With inbreeding variance among progeny means would increase while variance within progenies would decrease. Moreover, genetic analysis for seed size in interspecific crossing of soybeans suggested that the small seed was dominantly or partially dominant over the large seed (Williams, 1948), as a result in the succeeding generations the MS materials could maintain more large seeds, i.e., the MS would be an advantage to select large--seeded genotypes-, on the other hand, the PS would have a good chance to choose small--seeded genotypes in interspecific hybrids of soybeans.

The effects of seed size selection on other characters were showed in Table 2. It would be noted that regardless of selection methods, when progenies of two crosses were employed for larger seed selection, the plant height became shorter, stem diameter thicker, lodging decreased and yield traits improved. Furthermore, the genetic correlation analysis (Table 3) revealed that the correlation between seed size and each of plant height, branch number and lodging was most significantly negative, while the correlation between seed size and stem diameter as well as yield per plant was significantly positive. The analysis enhanced the ideal that seed size was an important character, which could impose high selection effects on others among interspecific crossing of soybeans.

Conclusion

The pedigree and mass selection methods of breeding for seed size were compared with F4 progenies in the two interspecific crossing of soybeans. The seeds of the mass selections were relatively larger than that of the pedigree selections in both large and small groups within two crosses, and the cases were also true with genetic variance for seed size. With used for larger seed selection, the plant height of progenies of two crosses became shorter, stem diameter thicker, lodging decreased and yield traits improved, informing that seed size selection is of importance in interspecific crossing of soybeans.

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Table 1. Comparison of selection effect on seed size between pedigree and mass methods in F4 progenies of two types of soybean cross.

Methods	Items	Types of cross			
		S17		G18	
		(1)+	(2)	(3)	(4)
PS	No. of plants	75	75	75	75
	100-seed weight	6.68 / 1.07++	4.49 / 0.70	9.02 / 1.49	6.85 / 0.61
	CV%	16.02	15.58	16.57	9.72
MS	No. of plants	75	75	75	75
	100-seed weight	7.28 / 1.52	4.86 / 0.89	9.41 / 1.79	7.00 / 0.71
	CV%	20.87	18.22	19.02	11.78
Comparison of methods	DBTSW+++	-0.61*/ 0.30	-0.37 / 0.28	-0.39 / 0.28	-0.15 / 0.21
	DBTCV++++	-4.85	-2.64	-2.45	-2.06

+ There were selection groups under crosses.
++ There was standard error following to mean 100–seed weight.
+++ Difference between two 100–seed weights.
++++ Difference between two CVs
* Significance at the 0.05 probability level.

Table 2 . The effects of seed size selection on other characters of F4 progenies of two crosses

Characters	S17				G18			
	PS		MS		PS		MS	
	(1)+	(2)	(3)	(4)	(1)	(2)	(3)	(4)
Plant height	128.81	137.65	121.72	133.89	116.14	129.59	111.77	126.91
Stem diameter	0.394	0.353	0.393	0.350	0.481	0.466	0.490	0.424
Branch number	4.50	6.13	4.31	5.96	4.13	4.19	4.01	5.43
Lodging number	3.13	3.84	3.81	4.01	3.31	3.48	3.40	3.57
Pods per plant	159.4	186.1	168.1	192.9	146.8	168.6	154.9	173.1
Seeds per plant	310.1	366.5	325.7	372.6	256.0	278.3	294.3	301.2
Yield per plant	21.28	17.16	22.61	18.76	25.91	22.04	25.39	23.88
Growth period	122.7	128.71	123.8	127.15	121.9	126.94	122.3	126.01

+ See the explanation of table 1.

Table 3. Correlation between seed size and some main characters of F4 progenies of two crosses from mass E nd pedigree selection methods

Type of cross	Selection methods	Growth Period	Stem diameter	Plant height	Branch number	Lodging index	Pods per Plant	Seeds per Plant	Yield per Plant
S17	PS	0.299*	0.235*	-0.258*	-0.323*	-0.204	-0.001	-0.124	0.105
	MS	0.119	0.370*	-0.335*	-0.025	-0.064	-0.214	-0.026	0.236*
G18	PS	0.263*	0.107	-0.312*	-0.085	-0.104	-0.220	-0.187	0.042
	MS	0.180	0.342*	-0.252*	-0.135	-0.218	-0.215	-0.204	0.069

* Significance at the 0.05 probability level.

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Identification of RAPD Markers Linked to Cercospora sojina Race 7 Resistance Gene in Soybean

Introduction

In recent years, marker-assisted selection (MAS) has received attention as a viable method for the improvement of major gene disease and insect pest resistance in crop plants. In contrast to the traditional selection based on phenotypic screening, molecular markers are refractory to environmental variation. Upon identification of molecular markers closely linked to desirable traits, marker-assisted selection can be performed for multiple resistance in early segregating generation and at early stage of plant development. Breeding disease-resistant genotypes using marker-assisted selection requires that (1) the resistance gene(s) be tagged by closely linked molecular markers, (2) the linkage be stable across generations and population and (3) an efficient way of screening large populations for molecular markers be available.

Our former study showed the resistance of NEAU9674 to Cercospora sojina race 7 in China is due to a single dominant gene (Zou et al., 1998). And the symbol of Rcsc7 was suggested to be assigned to the resistance gene. In this paper, we report on the identification of RAPD (random amplified polymorphic DNA) markers linked to Rcsc 7.

Materials and Methods

Genetic materials and disease evaluation are the same as those in the former study (Zou et al., 1998).

DNA was extracted from plants following slightly modification of the procedure of Dellaporta et al. (1984). Bulked segregating analysis (Michelmore et al., 1991) was performed by adding equal amount of DNA from 12 susceptible and resistance F2 individuals per bulk. Approximately 20 ng of genomic DNA template and 15 ng of single decamer primer (Operon Technologies) was used in a 25 µl reaction that contained 1 unit of Taq Polymerase (made in our lab), 10buffer, 2.0 mM $MgCl_2$ and 0.2 mM dNTPs, overlaid with 25 µl of mineral oil prior to amplification. Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler 480 programmed for five cycles of 1 min/ 94, 2 min/ 36, and 1 min/ 72, and then 35 cycles of 30 sec/ 94, 1 min/ 36and 1.5 min/ 72, followed by one final extension cycle of 10 min/ 72. Amplified DNA fragments were separated on 1.4% agarose gels using TAE buffer and visualized with Ethidium Bromide and UV illumination. A total of 850 primers has been screened between the two bulks.

Bands amplified by the RAPD primer and suspected to be allelic based on Mendelian segregation in the F2 population was tested for co-dominance using Southern hybridization. The bands from the possible co-dominant loci were cut from an agarose gel and the DNA was extracted using the GeneClean Kit (Bio101, Inc.). This DNA was labeled using the Multiprimer DNA labeling system of Amersham, and used to probe a Southern blot containing the bands amplified in the RAPD.

Linkage analysis was performed with the program Mapmaker 2.0. Kosambi function was selected. Linkage was considered significant if the LOD score was greater than 3.0.

Results and Discussion

The screening of 850 decamer primers against the R and S bulk resulted in the amplification of 4,420 discernible DNA fragments ranging from 2000 to 150bp, corresponding to an average of 5.2 fragments per primer. Of the primers screened, 11 generated a fragment that was present in the R bulk but not in the S bulk. When analyzed against the parents and the 93 F2 segregating individuals, three primers OPA04, OPQ12 and OPS03 generated polymorphic DNA fragments that were shown to be linked to the resistance gene. The order of these loci and their genetic distance were $OPQ12_{500}$ 17.3cM Rcsc7 8.7cM $OPS03_{620}$. 33.2cM $OPA04_{1100}$.

When amplified with primer OPS03, the polymophic band $OPS03_{620}$.and $OPS03_{580}$.were produced from the resistant parent(NEAU9674) and the susceptible parent(NEAU91212), separately. The two bands ($OPS03_{620+580}$) can be amplified from the F1 plants. And the three band types ($OPS03_{620}$, $OPS03_{580}$.and $OPS03_{620+580}$), observed in the F2 population, segregated in a ratio of 1 ($OPS03_{620}$.) : 2 ($OPS03_{620+580}$): 1 ($OPS03_{580}$.). $OPS03_{620}$.and $OPS03_{580}$.were excised from an agarose gel and hybridized to the other band through Southern hybridization. The result conformed that the two bands were allelic. The above results showed that it was a co-dominant RAPD marker. RAPD markers usually show dominant expression and are scored for the presence or absence of each amplified band. However, co-dominant expression of RAPD bands would be expected if an inserted or deleted sequence was found within the amplified region of some, but not all, of the individuals scored for each primer. Bands of different sizes, but with identical priming sites, would characterize similar in DNA sequence for all except the inserted or deleted section of the amplified region. Because of the co-dominant segregation of some RAPD fragments, these markers not only can be used like RFLP to distinguish heterozygotes and homozygotes, but also can be applied through simple and quick procedure. These markers should be more useful in the genetic mapping and marker-assisted selection than previously thought.

From Table 1, it can be seen that the accuracy of resistance prediction based on the co-dominant marker is high. Of the 27 individual plants that were scored as homozygous resistant based on the marker, all were resistance upon actual disease

scoring. This gives an accuracy of 100% (Table 1). Of the 41 individual plants that were scored as heterozygous resistant based on the marker, 40 were found to be resistance upon actual disease scoring. This gives an accuracy of 97.6% (Table 1). A low accuracy of 70.8% was obtained when predicting the susceptible individuals, because some F2 plants with susceptible band type($OPS03_{580}$) were resistance in the field evaluation. The low accuracy of susceptible prediction may be due to recombination events between the $OPS03_{620}$ and the Rcsc 7 locus, however, it should be considered that the low accuracy may be caused by the escaping of some F2 individuals from the inoculation in the field. We planed to evaluate the F3 progenies derived from the F2 individuals that were tested in 1997 to confirm the resistance or susceptible scored in 1997.

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Table 1. Accuracy of resistance prediction based on the co-dominant marker for the Rcsc7

Marker Prediction			Actual disease scoring		Accuracy of prediction
RAPD	No. of F_2 plants	R/Rr/rr	No. of R plants	No. of S plants	
$OPS03_{620}$	27	RR	27	0	100%
$OPS03_{620+580}$	42	Rr	41	1	97.6%
$OPS03_{580}$	24	rr	7	17	70.8%

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Inheritance of a Recently Discovered Soybean Virescent Mutant "NJ93V"

Introduction

Soybean chlorophyll-deficiency mutants have long been reported by researchers. Here, we report the inheritance of a probably new mutant recently discovered in Nanjing.

Source and description of the mutant

The virescent mutant was first discovered in 1993 in a synthesized recurrent population which was described by Song et al (1994), the colors of the cotyledon and leaves of the mutant are yellow. The yellow color remains to maturity. Color of stem is red. On average, each plant has about 40 pods, 4 branches and is about 60 cm high. The color of the mutant is stable from year to year and not affected by the temperature and light through five years field observation.

Inheritance of the mutant "NJ93V"

In 1994, crosses were made between mutant "NJ93V" and three entries, i.e. N69-2774 (the genotype for fertility is *Ms1 ms1*), T273H (the genotype for fertility is *MS3ms3)*, and NJ94-0915. "NJ93V" were used as female in each cross. In 1995, about 30 F1 hybrid seeds from each of the three crosses were planted in the field of Jiangpu Agricultural Experimental Station, Nanjing Agricultural University, Jiangpu, Jiangsu. All plants in each cross were normal green. It showed that the phenotype of virescence was not cytoplasmic-controlled. Meanwhile, ten F1 plants were harvested randomly from each cross, the seeds from each F1 plant were sowed in a single row in 1996. Number of virescent and normal green color plants were recorded and showed in Table 1. It showed that segregation of the three crosses fit 3:1 ratio in F2 populations.

Moreover, seeds of 10 virescent and about 20 of non-virescent plants from each F2 population were harvested individually, and seeds from a single plant were sowed in a single row in 1997. All F2 derived progenies of virescent plant in three crosses were virescent. Progenies of F2-derived non-virescent plants segregated. Ratios of non-virescent plants to virescent plants were 2:1(Table 2).

Results of bulked analysis of F2 non-virescent derived F3 lines with virescent plants were also shown in Table 3. Except the cross between NJ93V and N69-27740, the segregation of other two crosses fit 3:1.

From Table 1, Table 2, and Table 3, it observed that inheritance of virescent trait is controlled by one recessive gene.

Linkage of male-sterile gene with virescent gene

In 1996, F2 progenies from NJ93VxN69-2774, NJ93VxT273H were used to observe the linkage between *ms1, ms3* and virescent locus. 408 and 353 F2 plants were observed, the segregation were as listed in Table 5.

The linkage distance is not significantly different from 0.5. Thus gene controlling virescence is not linked to either *ms1 or ms3* genes.

Discussion

In 1995, 7 crosses were also made between "NJ93V" and varieties or lines: Xiansuidou, ATG, NJO93100, NJO93059, NJO93030, NJ93077 and NJ93007. The segregation of F2 population were listed in Table 6.

We also found that segregation of the virescence in some F2 populations: NJ93VxXiansuidou, NJ93VxATG, NJ93Vx NJO93030, and NJ93VxNJO93077 didn't fit 3:1 ratio. Different segregation ratios were observed when the mutant was crossed to different parents. Further observation through increasing sample size should be carried out. However, there is also possibility that the inheritance of the trait varied with crosses.

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Table 1. Number of virescent and non-virescent plants in F2 generation for each cross.

Cross	Non-virescent plants	Virescent plants	X2 (3:1)	P
NJ93VxN69-2774	306	102	0	
NJ93VxT273H	280	73	1.08	0.25-0.5
NJ93VxNJ94-09154	276	80	1.09	0.25-0.5

Table 2. Segregation of F2-derived F3 lines.

Cross	F2 virescent plants		F2 non-virescent plants		X2 (2:1)	P
	F3 virescent lines	F3 non-virescent lines	F3 virescent lines	F3 non-virescent lines		
NJ93VxN69-27740	10	0	13	7	0.01	>0.9
NJ93VxT273H	10	0	10	8	0.56	0.25-0.5
NJ93VxNJ94-09154	10	0	13	7	0.01	>0.9

Table 3. Number of virescent and non-virescent individuals in F2 non-virescent derived F3 progenies.

Cross	F3 non-virescent plants	F3 virescent plants	X2 (3:1)	P
NJ93VxN69-27740	342	139	5.19	0.01-0.025
NJ93VxT273H	279	72	3.53	0.05-0.10
NJ93VxNJ94-09154	341	93	0.05	>0.9

Table 5. Linkage between male sterility and virescence.

Crosses	Male Sterile		Male Fertile		r*
	Non-virescence	Virescence	Non-virescence	Virescence	
NJ93VxN69-27740	42	18	264	84	0.50
NJ93VxT273H	26	10	254	63	0.46

* r: recombination value.

Table 6. Number of virescent or non-virescent plants in F2 generation for each cross.

Cross	Non-virescent plants	Virescent plants	X2 (3:1)	P
NJ93VxXiansuidou	255	124	12.5	<0.05
NJ93VxATG	316	14	79.2	<0.05
NJ93VxNJO93100	197	72	0.4	>0.5
NJ93VxNJO93059	272	86	0.1	>0.75
NJ93VxNJO93030	297	46	23.9	<0.05
NJ93VxNJO93077	158	79	8.3	<0.05
NJ93VxNJO93007	196	68	0.05	>0.75

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Inheritance of Resistance of NEAU9674 to Race 7 of Cercospora sojina in Soybeans in China

Introduction

Frogeye leafspot of soybean caused by Cercospora sojina Hara is a kind of worldwide disease (Athow and Probst, 1952). It has become a major soybean disease problem and production constraint in the Northeast of China, especially in Heilongjiang Province. Among the eleven races of Cercospora sojina identified in Heilongjiang province, race 1, race 7 and race 10 were found to be the main races (Huo et al, 1988). The use of resistant cultivars can greatly reduce the incidence of this disease. NEAU9674, developed by Northeast Agricultural University, was reported to be resistant to all physiological races collected in China and was widely used in the soybean resistant breeding (Yang et al, 1996). In this paper, we report on the inheritance of resistance of NEAU9674 to race 7 of Cercospora sojina in China.

Materials and Methods

A cross of NEAU9674 (resistant to race 7) symbol 180 \f "Symbol" \s 10.5×} NEAU91212 (susceptible to race 7) was made in 1995. F1 seeds were planted in Hainan Island in the winter, 1995. Two parts of F2 plants were planted in 1996 and 1997, separately. Progenies of resistant and susceptible F2 plants planted in 1996 were advanced to F3 plant rows to verify the F2 segregation. Thirty seeds were planted in each F3 row in 1997. Both parents and F1 were included in 1996 and 1997.

Cultures of C. sojina were maintained and inoculum was produced on a medium composed of sterilized sorghum seeds. Conidial suspensions were made by flooding the fungus growing on the surface of sorghum seeds with sterile water. The suspensions were passed through several layers of cheesecloth to remove large mycelia fragments. Condidal suspensions were adjusted to a concentration of 5 symbol 180 \f "Symbol" \s 10.5×} 10^3 spores per milliliter. All plants were inoculated by spraying with spore suspension in July before uninoculated soybeans in the area showed any symptoms of frogeye leafspot. Since these plants could not be placed in a moist chamber, they were inoculated late in the day. Water was sprayed to the plants after the day of inoculation to improve the humidity in the field. The number and size of lesions were recorded 14 days after inoculation. The plants with either no lesions or no more than three small leisions (< 0.4mm in diameter) were scored as resistance. And the plants with large spreading lesions or more than three small leisions were recorded as susceptible.

Experimental Results

F2 segregation and F3 progenies breeding behavior were shown in Table 1. A satisfactory fit a ratio of 3 resistant: 1 susceptible was obtained in the F2 progenies of the cross. The progenies of the resistant F2 plants were in a ratio of 2 heterozygous to 1 homozygous resistant in this cross. The progenies of all susceptible F2 plants tested were homozygous susceptible. The data indicate that resistance of NEAU9674 to race 7 in China is due to a single major dominant gene. The symbols of Rcsc 7, rcsc 7 are suggested to be assigned to this gene pair. (Rcs_ has been assigned to the gene, which is resistant to Cercospora sojina race_ identified in USA. Rcsc_ were suggested to be assigned to the gene resistant to race_ identified in China).

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Table 1. Breeding behavior of parents, F1, F2 and F3 progeny to race 7 of Cercospora sojina

Generation	Number of plants or rows		R: S	Expected ratio	χ^2	P
	Resistant	Susceptible				
NEAU9674(1996,1997)[a]	100	0				
NEAU91212(1996,1997)	0	100				
F_1(1996,1997)	100	0				
F_2(1996)[b]	52	18	3.00:1	3:1	0.019	0.75-0.90
F_2(1997)[c]	75	18	4.16:1	3:1	1.581	0.10-0.25
F_2(1996,1997)	127	36	3.52:1	3:1	0.719	0.50-0.75
$F_{2S:3}$[d](1997)		18				
$F_{2R:3}$[e](1997)	33	17	1.94:1	2:1	0.010	0.90-0.95

a) Results of 1996 and 1997;
b) Results of 1996;
c) Results of 1997;
d) F3 progeny from F2 susceptible plants;
e) F3 progeny from F2 resistant plants.

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Inheritance of Tofu Output in the Cross Guanyundaheidou X Liuhexiaoyeqing

Introduction

Tofu output is one of the important target traits in soybean breeding. Gao and Gai (1997) reported a method of determination of tofu output on a single seed base. Wang and Gai (1997) reported the approach of identification of major-polygene mixed inheritance of quantitative traits and estimation of genetic parameters. The above discoveries make it possible to study the inheritance of tofu output on a single seed base.

Materials and Methods

One hundred and twenty six $F_{2:3}$ embryo generation families (seeds from F_2 plants) and 40 $F_{2:3}$ plant generation families (seeds from F_2 lines, which were also $F_{2:4}$ embryo generation families) were sampled for analysis. The embryo generation families were analyzed for their tofu output by using single-seed method. The plant generation families were analyzed for their tofu output by using minor-specimen method with which 30g seeds per plant were used.

Results and Discussion

In $F_{2:3}$ embryo generation and $F_{2:3}$ plant generation, there existed obvious genetic variation for both dry and fresh tofu output, especially for the latter. In $F_{2:3}$ embryo generation, the generation mean of dry tofu output tended to the high parent (Liuhexiaoyeqing), while that of the fresh tofu output tended to the low parent. The heritability values for dry and fresh tofu output were 57.9% and 70.2%, respectively (Table 1 and2).

Results of the inheritance study (Table 3) showed that tofu output was controlled by both one major gene and polygenes, modified with environments. The major gene of high dry tofu output was completely dominant and the major gene heritability was 45.9%. The fresh tofu output appeared to be of negative and partial dominance, its heritability was 69.7%. The polygene heritability values were 12.03% for dry tofu output and 0.5% for fresh tofu output, respectively.

In $F_{2:3}$ plant generation, the similar results were obtained for dry tofu output, but for fresh tofu output, two major genes might be involved (Table 3).

Results from $F_{2:3}$ embryo generation and from $F_{2:3}$ plant generation were different for fresh tofu output, which may due to 1) limited sample size of $F_{2:3}$ plant generation, 2) difference between micro-specimen and minor-specimen analysis, and 3) the deficiency of the genetic model. Further study on inheritance of tofu output is being carried out.

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Table 1. Distributions of dry tofu output in $F_{2:3}$ embryo and plant generation in the cross Guanyundaheidou X Liuhexiaoyeqing (g/100g seeds).

Tofu	Embryo generation			Plant generation
Output	$F_{2:3}$	P_1	P_2	$F_{2:3}$
28.8 - 30.8	5			2
30.8 - 32.8	2			
32.8 - 34.8	1	2		1
34.8 - 36.8	5			4
36.8 - 38.8	13	1		1
38.8 - 40.8	14		1	6
40.8 - 42.8	14	1	1	6
42.8 - 44.8	22	1	2	3
44.8 - 46.8	22		1	9
46.8 - 48.8	16			3
48.8 - 50.8	9			2
50.8 - 52.8	2			1
52.8 - 54.8	1			
>54.8				2
n	126	5	5	40
$\bar{x}$	42.9	38.4	42.7	43.3
s^2	24.43	12.97*	7.60*	24.32

* The variance was adjusted to a five seeds per plant basis which was the sample size of each $F_{2:3}$ family. The same is true for the next table.

Table 2. Distributions of fresh tofu output in $F_{2:3}$ embryo and plant generation in the cross Guanyundaheidou X Liuhexiaoyeqing (g/lOOg seeds).

Tofu	Embryo generation			Plant generation
Output	$F_{2:3}$	P_1	P_2	$F_{2:3}$
<209.9				14
209.9 - 231.9	3			6
231.9 - 253.9	4			3
253.9 - 275.9	10			1
275.9 - 297.9	16	1		6
297.9 - 319.9	18			5
319.9 - 341.9	23	3	1	4
341.9 - 363.9	22	1		1
363.9 - 385.9	12			
385.9 - 407.9	7		1	
407.9 - 429.9	8		2	
429.9 - 451.9	1		1	
451.9 - 473.9	1			
473.9 - 495.9	1			
n	126	5	5	40
$\bar{x}$	331.7	327.3	363.5	244.1
s^2	3716.9	589.7*	1629.5*	3693.7

Table 3. Gene effects and genetic parameters in $F_{2:3}$ embryo generation and $F_{2:3}$ plant generation in the cross Guanyundaheidou X Liuhexiaoyeqing.

Trait	Generation Type	Number of major gene	Major gene			Degree of dominance	Heritability of major gene	Heritability of polygene
			Ratio	Additive effect	Dominance effect			
Dry tofu output	Embryo	1	1:3	4.1	4.19	1	45.9	12.03
	Plant	1	1:3	4.6	4.62	1	56.0	—
Fresh tofu output	Embryo	1	1:2:1	68.09	-21.19	-0.3	69.7	0.5
	Plant	2	9:7	—	—	—	—	—

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Major Plus Minor Gene Mixed Inheritance of Resistance of Soybeans to Agromyzid Beanfly *(Melanagromyza sojae* Zehntner)***

Key words: soybean, Agromyzid beanfly *(Melanagromyza sojae* Zehntner), inheritance of resistance to insects, major plus minor gene mixed inheritance model

The soybean agromyzid fly, *Melanagromyza sojae* Zehntner, is one of the important pests in soybean production in the south region of the Great Wall of China. It infects almost 100% of soybean plants and has caused great yield losses every year. Breeding for new cultivars with the resistance to the beanfly is the most effective way to control its damage. Wei et al (1989) made crosses between resistant lines and susceptible lines and investigated the number of insects in the stem (NIS) and the total number of insects (NIP, which is NIS plus the number of insects in petiole). They classified individuals in F_2 and $F_{2:3}$ families into resistant ones or susceptible ones by finding a common critical value between two peaks of a segregating distribution. From the segregation ratio of resistance and susceptibility, they concluded that the resistance was controlled by one dominant gene, and no cytoplasmic effect was found. Due to the quantitative variation occurred to the phenotypes, they guessed but could not convince that the resistance might be modified by some polygenes.

For those quantitative traits showing major gene effects and also having continuous variations, it is supposed that both major genes and polygenes are included in the genetic system. Wang (1996) and Wang & Gai (1997, 1998a,b) developed the segregation analysis method to identify the mixed inheritance model of plant quantitative traits and estimate related genetic parameters. The principle of the method can be described as follows. Firstly, it is supposed that each segregating population is a normal mixture of major gene component distributions modified by polygenes and environments. Secondly, the major gene heritability and polygene heritability are defined. Thirdly, likelihood functions under various possible genetic models are established, maximum likelihood estimates of parameters contained in each model are calculated through EM algorithm, and the most fitted genetic model and its parameter estimates are chosen by Akaike's information criterion, likelihood ratio test and tests of fitness. And finally, each individual is classified into suitable component distribution by using Bayesian posterior probabilities. They have developed method for individual segregating populations such as F_2, backcrosses and $F_{2:3}$ (Wang & Gai, 1997). Based on that, the joint segregation analysis of multiple generations is developed. Considering the difficulty degree of crossing successfully, joint analysis based on the five population P_1, F_1, P_2, F_2, $F_{2:3}$ (Wang & Gal, 1998b) and the six population P_1, F_1, P_2, B_1, B_2, F_2 (Wang, 1996) are developed separately.

In the present paper, the joint segregation analysis of multiple generations of P_1, F_1, P_2, F_2 and $F_{2:3}$ is used to re-analyze the genetic data in Wei et al (1989), with the objectives to identify whether the inheritance to the beanfly is controlled by only one major gene or mixed a major gene and minor genes, and furthermore, to estimate the genetic parameters related to both major gene and minor genes.

Materials and methods

Three crosses were made between resistant line JNCND, WXCJGJ, 1138-2 and susceptible line PXTED, HJQDHY (Table 1). The resistant indices were the number of insects in the stem (NIS) and the total number of insects (NIP). An experiment design similar to split plot design was used in the experiment, with crosses in main plot and parents, hybrid generations in sub-plot. The genetic data were from all the five populations of P_1, F_1, P_2, F_2 and $F_{2:3}$, and the method by Wang and Gal (1998b) were used.

Results

Distribution characteristics of NIS

Both NIS and NIP can be used as index for the resistance to beanfly. But NIS is of higher correlations between years, lower error variances, higher heritability values than NIP. So Gai & Cui (1989) proposed to use NIS as the major index for the resistance.

The frequency distributions of NIS in various populations are listed in Table 1. Variation in homogeneous population P_1, P_2 and F_1 can be viewed as the environmental variation. F_1 has a tendency toward resistant parent. F_2 and $F_{2:3}$ are genetically segregating populations, but the variation of F_2 is greater than that of $F_{2:3}$ except cross II. The distributions of NIS of F_2 in all the three crosses demonstrate single mode, but those of $F_{2:3}$ demonstrate bi-modality. The component distribution with a lower mean occupies a larger proportion in the mixture than that with a higher mean does.

Major plus minor gene mixed inheritance model for the resistance of soybeans to the fly

Firstly, maximum likelihood, AIC value and maximum likelihood estimates in every model were calculated by using maximum likelihood method and EM algorithm. Table 2 gives the results of model D, the best fitted model according Akaike's information criterion. Here, AIC = $-2L_c(\Phi)+2N$, where $L_c(\Phi)$ is the maximum loganithm likelihood, N is the number of independent parameters. Model D represents mixed one major gene and polygenes, and there may exist interaction among polygenes. So the results in the paper extend those reported by Wei et al (1989).

In Table 2, μ_1, μ_2 and μ_3 stand for the population means of P_1, F_1, and P_2 respectively, and σ^2 for the combined variance of the three homogeneous populations. μ_{41}, μ_{42} and μ_{43} stand for

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means of component distributions in F_2, and σ_4^2 for the common variance of components in F_2. μ_{51}, μ_{52} and μ_{53} stand for means of the three kinds of families derived from the three F_2 major genotypes respectively, σ_{51}^2 for the variance of the components having mean μ_{51} and μ_{53}, and σ_{52}^2 for the variance of the component having mean μ_{52}. Let A-a represent the two alleles of the major gene locus, then, μ_{41}, μ_{42} and μ_{43} are the means of AA, Aa and aa components in F_2 respectively, and μ_{51}, μ_{52} and μ_{53} the means of families derived from AA, Aa and aa F_2 individuals. From the first order position parameters in Table 2, it is obvious that μ_{41} is very close to μ_{42}, and μ_{51} is very near to μ_{52}. This illustrates the component distribution AA and Aa overlaps seriously. The situation is the same for the two indices in the three crosses.

Major gene and polygene effects of the resistance of soybeans to the fly

In Table 2, σ^2, which is the weighted average of variances of population P_1, F_1, and P_2, can be used to estimate the environmental variance in segregating populations, i.e., σ^2 can be looked as the environmental variance in F_2 and σ^2/n (n is the sample size of a $F_{2:3}$ family) the environmental variance for $F_{2:3}$ family mean population. σ_4^2 is the variation in F_2 except major gene variation, so, if $\sigma_4^2 \geq \sigma^2$, $\sigma_4^2 - \sigma^2$ can be looked as the estimate of the polygene variance in F_2; otherwise, the polygene variance in F_2 is set to 0. σ_{51}^2 is the variation in $F_{2:3}$ except major gene variation, so, if $\sigma_{51}^2 \geq \sigma^2/n$, $\sigma_{51}^2 - \sigma^2/n$ can be looked as the estimate of the polygene variation in $F_{2:3}$; otherwise, the polygene variance in $F_{2:3}$ is set to 0. Some estimates of polygene heritabilities are estimated as 0, but there exist polygene variations from the model test. There might be two reasons for this case. One is that it is not true to look σ^2 as the estimate of the environmental variation segregating populations. The other is that there may exist epistasis effects between the major gene and polygenes. d, h, [d] and [h] in Table 3 are estimated by least square method from the following equations.

$\mu_1=m+d+[d]$, $\mu_2=m+h+[h]$, $\mu_3=m-d-[d]$,
$\mu_{41}=m+d+(1/2)[h]$, $\mu_{42}=m+h+(1/2)[h]$, $\mu_{43}=m-d+(1/2)[h]$,
$\mu_{51}=m+d+(1/4)[h]$, $\mu_{52}=m+(1/2)h+(1/4)[h]$, $\mu_{53}=m-d+(1/4)[h]$

where d and h are the additive and dominance effects of major gene, and [d] and [h] arc the additive and dominance effects of polygenes.

From Table 3, the additive effects of major gene are -2.00 ~ -1.71 heads/plant, and the degrees of dominance are 1.05 ~ 1.39. The additive and dominance effects of minor genes were generally less than those of major gene and varied among crosses indicating different minor gene systems. The major gene heritability values in F_2 were 44.2 ~ 72.9%, those in $F_{2:3}$ were 93.1 ~ 95.0%, which are greater than those in F_2. The minor gene heritability values in F_2 were 0 ~ 13%, those in $F_{2:3}$ were 0 ~ 0.5%, which are less than those in F_2. Additive effect ([d]) and dominance effect ([h]) of polygenes can be positive or negative among crosses, which shows the genetic background of polygenes is different among crosses. But, in general, the additive and dominance effect of polygenes are less than those of the major gene.

Classification of major gene genotype in segregating populations

While the inheritance model is selected, the posterior probabilities of each F_2 individual and $F_{2:3}$ family belonging to its respective component can also be calculated at the same time. So F_2 and $F_{2:3}$ segregating populations can be classified by Bayesian rules. The classificating process is omitted here, and results were listed in Table 4. In Table 4, the ratio of Aa+AA to aa classified by posterior probabilities conform to the ratio 3:1 except F_2 in cross II. From the results of classification, the critical value between resistance and susceptibility can be arrived at. For trait NIS, the unique line was acquired, i.e., those with NIS equal or less than 3 head/plant in F_2 can be looked as resistant plants and those with NIS greater than 3 the susceptible plant.

Conclusions and discussion

The following conclusions are made further clear. (1) Inheritance of the resistance indicated by NIS can be fitted in the mixed one major gene and polygene model. (2) Resistance is almost completely dominant with the degree of dominance 1.05 ~ 1.39. (3) The additive effect in each cross is similar to each other, with the range -2.00 ~ -1.71 heads/plant. The difference might be due to the distinct in background of polygenes. (4) Additive effect ([d]) and dominance effect ([h]) of polygenes can be positive or negative among crosses, which shows the genetic background of polygenes is different among crosses. But, in general, the additive and dominance effect of polygene are less than those of the major gene. (5) Major gene heritability values in F_2 are 44.2% ~ 72.9%, and in $F_{2:3}$ are 93.1% ~ 95.0%. Although polygenes are included in the inheritance system, they contribute only a little to the phenotypic variation. Polygene heritability values in F_2 are 0 ~ 13.0%, and in $F_{2:3}$ are 0 ~ 0.5%. (6) F_2 individuals and $F_{2:3}$ families can be classified into major gene genotypes through Bayesian rules from posterior probabilities. From the classification, the critical line of component distribution can be arrived. The common critical value ($3<x<4$) was acquired in the three crosses for trait NIS.

Results in this paper indicated that more information could be obtained from the major-polygene mixed genetic analysis. By replacing traditional genetic analysis method (to find a critical value and conclude from the segregation ratio) with the new method, some subjective factors in obtaining segregation ratio can be avoided. So, more objective results can be achieved. Genetic data in joint segregation analysis consist of plant-level data from P_1, F_1, P_2 and F_2 and family-level data from $F_{2:3}$ families. Generally speaking, family data are more precise than plant data because less environmental errors are included in the family means. But joint analysis compromises such precision, i.e., the precision is improved for F_2 but decreased for $F_{2:3}$. So it is possible to draw more precise conclusion from only $F_{2:3}$ population. However, from the joint analysis, more genetic parameters are estimable. Therefore, it can be easily accepted. In order to improve the overall precision, the errors in experiment should be well-controlled for plant-level data in the experiment.

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Table 1. Frequency distribution of the number of insects in stem in various generations of soybean

Cross	Generation	0	1	2	3	4	5	6	7	8	9	n	$\bar{x}$	S^2
I	P_1	3	7	4	6							20	1.65	1.13
JNCWD x HJQDHY	F_1	4	4	6	6							20	1.70	1.21
	P_2						4	6	3	3	4	20	6.85	2.03
	F_2	26	36	45	34	25	12	5	2	1	3	189	2.47	3.46
	$F_{2:3}$	7	61	5		9	17	1				100	2.36	2.99
II	P_1	4	7	7	2							20	1.35	0.83
WXCJGJ x PXTED	F_1	1	11	5	3							20	1.50	0.65
	P_2					5	3	6	4		2	20	5.85	2.23
	F_2	18	52	28	14	11	10	8	5			146	2.24	3.54
	$F_{2:3}$	5	52	5		5	15	9				91	2.71	3.90
III	P_1					6	6	5	2	1		20	1.90	1.19
PXTED x 1138-2	F_1	5	7	5	3							20	1.30	1.01
	P_2	2	5	5	8							20	5.30	1.31
	F_2	24	36	39	37	20	20	12	9	3		200	2.82	4.10
	$F_{2:3}$	16	68	15		5	18	5				127	2.26	3.08

Table 2. The most fitted genetic model, AIC value and estimated parameters of the component distribution of the resistance of soybean to beanflies

Cross	Model	AIC	μ_1	μ_2	μ_3	μ_{41}	μ_{42}	μ_{43}	μ_{51}	μ_{52}	μ_{41}	σ^2	σ_4^2	σ_{51}^2	σ_{52}^2
I	D	1220.75	1.65	1.70	6.85	1.82	1.83	4.84	1.30	1.36	5.13	1.48	1.93	0.15	0.58
II	D	1029.10	1.35	1.50	5.85	1.32	1.37	5.16	1.39	1.47	5.54	1.26	0.96	0.27	0.95
III	D	1364.05	1.90	1.30	5.30	2.25	1.72	5.49	1.30	1.41	5.44	1.19	1.59	0.18	0.79

Table 3. The estimated genetic parameters of resistance of soybeans to beanflies from a joint analysis of P_1, P_2, F_1, F_2 and $F_{2:3}$

Generation	Estimate	Cross I	Cross II	Cross III
All	d	-1.71	-2.00	-1.85
	h	-1.79	-2.20	-2.57
	h/d	1.05	1.10	1.39
	[d]	-0.88	-0.25	0.15
	[h]	-0.49	0.14	0.65
F_2	σ_p^2	3.46	3.54	4.10
	h^2_{mg}(%)	44.2	72.9	61.2
	h^2_{pg}(%)	13.0	0	9.8
$F_{2:3}$	σ_p^2	2.99	3.90	3.08
	h^2_{mg}(%)	95.0	93.1	94.2
	h^2_{pg}(%)	0	0.5	0

Table 4. The estimated F_2 genotypes of number of insects in stem from a posterior probability analysis on F_2 and $F_{2:3}$

Cross	Generation	Number of insects	Number of plants (lines)	Estimated genotype
I	F_2	0–3	141	Aa+AA
		4–9	48	aa
	$F_{2:3}$	0.6–2.6	73	Aa+AA
		4.4–6.0	27	aa
II	F_2	0-3	112	Aa+AA
		4-7	34	aa
	$F_{2:3}$	0.8–4.6	65	Aa+AA
		4.8–6.6	26	aa
III	F_2	0–3	136	Aa+AA
		4–8	64	aa
	$F_{2:3}$	0.0–2.8	99	Aa+AA
		4.8–6.6	28	aa

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Post-flowering Photoperiod Response of Soybean Varieties with Similar Flowering Dates and Different Maturity Dates*

Introduction

Soybean is a short day plant. It was well-known that late flowering varieties were more sensitive to photoperiod than the early ones because they needed shorter critical daylength to induce flowering (Garner and Allard, 1920).

Among the varieties with similar flowering dates, there were great differences in maturity dates, this meant that these varieties were similar in flowering dates and different in maturity dates (Wang, 1981; Liu et al, 1989). Until now, little was known about whether the length of time from flowering to maturity was related to the sensitivity of photoperiod response after flowering. In this study, typical varieties screened from thousands of germplasm accessions in south China were used and different photoperiod treatments were conducted to answer this question.

Materials and Methods

Eight spring-sown soybean varieties (Table 1) and thirteen summer-sown varieties (Table 2) were grown in Nanjing (32° N) in 1995 and 1996. The spring-sown varieties were planted on April 22 and the summer-sown varieties were planted on June 15 in both years. After flowering (R1), the following photoperiod treatments were conducted: (1) natural photoperiod in Nanjing; (2) short day of 12h; (3) long day of 15h. The methods of photoperiod treatments were as our former report (Han and Wang, 1995), developmental stages were determined following Fehr et al (1977). Agronomic characters were also recorded. Maturity hastening rate (MHR) was introduced as an index of photoperiod sensitivity.

MHR(%)=((Days from R1 to R7 in 15h) - (Days from R1 to R7 in 12h))/(Days from R1 to R7 in 12h)×100%.

Results and Discussion

The varieties belonged to the same ecotypes (spring-sown or summer-sown in original sites) in this study were similar in flowering dates, but they varied in maturity dates. In natural photoperiod of Nanjing, the range of days from emergence to beginning flowering (R1) of spring and summer-sown varieties were 5.5 and 6.5, and the range of days from R1 to R7 (physiological maturity) was 18.4 and 34.0 (Table 1 and Table 2), respectively. The correlation analysis (Table 3) showed that both in spring- and summer-sown varieties, the length from R1 to R7 in natural photoperiod was positively related to MHR, it meant that the longer the reproductive growth period, the more sensitive to post-flowering photoperiod. For all the varieties tested, reproductive duration was significantly shortened by short day treatment, demonstrating that the post-flowering photoperiod response was quite sensitive.

Among the varieties tested, spring-sown varieties of Wujinhongchadou and Suofu 307 (introduced from America), and summer-sown variety of Baohexuan 3 had lower MHR value than the varieties with similar flowering dates and maturity dates, showing that they were less sensitive to post-flowering photoperiod.

The length of reproductive period was positively related to plant height in two ecotypes (Table 3). In spring-sown varieties, no significant relationship between the days from R1 to R7 and yield components was found. In summer-sown varieties, the length of reproductive period was positively related to 100-seed weight ($P<0.01$), but it was slightly negatively related to seed yield per plant. In this study, most of the varieties were landraces or old varieties and the growth season in Nanjing was long enough. If the highly developed varieties were used and in short-season regions, the relation between reproductive duration and agronomic characters might be different from the results above.

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Table 1. Post-flowering photoperiod response of spring-sown soybean varieties with similar flowering dates and different maturity dates[1]

Variety	VE-R1[2] under natural photoperiod (d)	R1-R7 (d)			Maturation hastening rate[4] (%)	(R1-R7)/(VE-R1) under natural photoperiod
		NPP[3]	12h	15h		
Huangyanwuyuebai	45.3	44.6	40.5^{B}	48.3^{A}	16.1	0.99
Wujinhongchadou	48.0	45.2	39.7^{B}	44.7^{A}	11.2	0.94
Fulingzaoquingdou	46.2	45.8	38.2^{B}	45.8^{A}	16.6	1.00
Rugaowuyuekuiding	49.4	46.3	41.2^{B}	50.2^{A}	17.9	0.94
Suofu 307	44.1	50.2	44.3^{B}	50.0^{A}	11.4	1.18
Shexiansucunheidou	47.1	51.8	46.2^{B}	54.5^{A}	15.2	1.16
Siyueba × Wuyueba	47.8	57.0	43.5^{B}	60.2^{A}	27.7	1.22
Shangyukaishanbai	48.4	63.0	45.7^{B}	73.2^{A}	37.6	1.29
Average	**47.0**	**50.5**	**42.4^{B}**	**53.4^{A}**	**19.2**	**1.09**

[1] The means of the days from R1 to R7 within a row without the same letter are significantly different (P<0.01)
[2] VE—Emergence; R1—Beginning bloom; R5—Beginning seed; R7—Physiological maturity
[3] NPP—Natural photoperiod
[4] MHR (maturity hastening rate, %) = (Days from R1 to R7 in 15h) - (Days from R1 to R7 in 12h))/ (Days from R1 to R7 in 15h) × 100%

Table 2 Post-flowering photoperiod response of summer-sown soybean varieties with similar flowering dates and different maturity dates[1]

Variety	VE-R1[2] under natural photoperiod (d)	R1-R7 (d)			R1-R5 (d)		Maturation hastening rate[2] (%)	(R1-R7)/(VE-R1) under Natural photoperiod
		NPP	12h	15h	12h	15h		
Xuyonghongdou	46.4	51.8	46.4^{B}	75.0^{A}	20.0^{B}	32.2^{A}	37.9	1.12
Qianshankunlongqingdou	45.3	53.6	42.0	not matured	19.0^{B}	57.5^{A}	67.0	1.18
Bohexuan-3	47.7	54.2	48.3^{B}	67.7^{A}	19.8^{B}	29.6^{A}	33.1	1.14
Liupanshuichidou	45.8	57.8	49.8^{B}	83.8^{A}	24.0^{B}	38.2^{A}	37.2	1.26
Wangjianghuangdou	44.9	60.8	49.2	not matured	22.9^{B}	70.4^{A}	67.5	1.35
Yunxianludou	45.9	68.2	55.5	not matured	20.5^{B}	46.4^{A}	55.8	1.49
Dongguanqingxihuangdou	44.1	70.0	51.5	not matured	21.0^{B}	81.1^{A}	74.1	1.59
Nannong1138□2	43.4	71.5	55.3	not matured	21.6^{B}	78.8^{A}	72.6	1.65
Baiwudou	43.0	71.7		not matured	24.0^{B}	77.2^{A}	68.9	1.67
Zhengyuanmengdalupidou	46.4	75.9	54.0	not matured	25.6^{B}	71.5^{A}	64.2	1.64
Batanghuangdou	45.1	78.0	66.8	not matured	25.4^{B}	93.4^{A}	72.8	173
Ruilishanghaiqingdou	48.4	79.5	66.0	not matured	22.8^{B}	61.7^{A}	63.0	1.64
Ludingheidouzi	49.5	85.8	69.2	not matured	27.7^{B}	71.7^{A}	61.4	1.73
Average	**45.8**	**67.6**	**54.5**	**>75.5**	**22.6^{B}**	**62.3^{A}**	59.6	**1.48**

[1] See the footnoles of Table 1.
[2] MHR was calculated with the days from R1 to R5.

Table 3. Coefficients of correlation between the days of reproductive period (R1—R7) and the agronomic characters of soybean varieties with similar flowering dates and different maturity dates

Ecotype	Maturation hastening rate (%)	Plant height	Seed number per plant	Seed yield per plant	100-seed wt.
Spring-sown R1-R7	0.5215**	0.3217**	-0.0520	-0.0694	0.0571
Summer-sown R1-R7	0.5457**	0.4124**	-0.1644	-0.0007	0.3791**

**: P<0.01

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Preliminary Identification of a RAPD Marker Linked to the Null Gene *Titi* of Kunitz Trypsin Inhibitor Using Near-Isogenic Lines

Abstract

420 10-mer oligonucleotide primers were used for screening RAPD markers for soybean Kunitz trypsin inhibitor (SKTi) in 'Williams' near-isogenic lines (NILs). A specific 560bp band was amplified with primer OPU-09 from NILs carrying *titi* locus. The linkage was tested by RAPD-PCR method using OPU-09 as a primer and genomic DNA isolated from leaves of six different F_5 lines and 40 randomly selected seeds from a soybean cultivar (92NK08 harbouring *titi* gene,F_{10}) as template. The marker's linkage was further confirmed by enzymatic assay, the Native-PAGE with same plant materials. The results of RAPD analysis and enzymatic assay are correlated. It indicates that this RAPD marker and quick PCR assay can be implemented in the breeding program for soybean nutritional improvement.

Key words: soybean, Kunitz trypsin inhibitor, RAPD, near-isogenic line (NIL)

Introduction

Soybean Kunitz trypsin inhibitor (SKTi) is a major antinutritional factor that inhibits the utilization of soybean protein in human diet. It is known that three dominance alleles, Ti^a, Ti^b, Ti^c and one null allele, *titi*, are existed on the soybean chromosomes. Recently, Zhao (1992) reported a new dominant allele, Ti^d. To date, the null genotype *titi* has not been found in Chinese soybean cultivars (Wang et al., 1986). Ding (1992) transferred the *titi* gene into Chinese soybean lines by sexual hybridization followed by intensive selection.

The development of genetic map (molecular markers) for soybean is relatively slow compared with that in other economically important crops. This has been due largely to inherent difficulties in performing successful sexual crosses, a lack of cytogenetic markers and the unavailability of proper genetic stocks (R. C. Shoemaker et al. 1996). In this paper, we describe the development of a RAPD marker that links to the *titi* gene using a near isogenic lines (NIL) as a material.

Materials and Methods

Plant materials

The donor parent of 'Williams' NIL with *titi* (L83-4387), a recurrent parent, Ludou No. 4 and the offspring that had been backed cross for 7 generations were used in this study. 'Williams' NIL with Ti^b and Ti^c genotype were also included for comparisons. For conformation of the linkage between selected RAPD markers and the *titi* allele, genomic DNA isolated from leaves of six different crosses (F_5) in 1997 namely Zhongdou 19 × ti(L81), ti(Ludou No.4 × L83) × ti(Yudou8 × L81), Anbian × ti(L83), Ludou No.4 × ti(L83), ti × lox2 and 3, ti × lox2 and 40 randomly selected seeds of a cultivar(92NK08, F_{10}) were analyzed by RAPD and by Native-PAGE.

RAPD analysis procedure

Total gnomic DNA was extracted from fresh young leaves collected in bulk either in the field or in the greenhouse. DNA extraction was performed according to the method described by Davis et al. (1986). DNA amplification was conducted in PCR amplifier PE9600 as following condition: 94°C for 3 min, then at 94°C for 15s, 38°C 30s, 72°C 45s in a cycle, totally 42 cycles, following the last cycle extension at 72°C for 5 min. Polymerase (Taqase) and buffer were purchased from Taq Gene co. . Reaction products were electrophoresed in 1.5% agarose gels at 5v/cm for 3 hours.

SKTi enzymatic assay

Semi-seeds of six F_5 lines and 40 semi-seeds of a cultivar (92NK08) were analyzed by Native-PAGE to distinguish the absence of SKTi (Ding et al., 1990).

Cloning a band of interested

Single EB-staining band removed from agarose was purified and reamplified following the procedure described above except that the annealing temperature was increased 4°C. This reamplified band was cloned into pGEM-5Zf vector (purchased from promega co.). The cloned fragment was identified by RAPD-PCR with the procedure described above using plasmid DNA as template and OPU-09 as a primer, and by enzymatic digestion using endonuclease.

Results

1. Out of 420 10-mer oligonucleotide primers, OPU-09 amplified a 560bp band appeared specifically in *titi*.

2. The results of RAPD analysis of leaves of six F_5 lines harboring the *titi* gene and 40 seeds of cultivar 92NK08 carrying the *titi* gene are perfectly fit to the results of enzymic analysis, indicating that the marker (560bp product) is linked to the *titi* gene.

3. RAPD-PCR amplification and Pst1, AatII digestion showed that the inserted band in the pGEM-5Zf vector has the same molecular weight as the RAPD marker ($OPU\text{-}09_{560}$).

Discussion

The Kunitz trypsin inhibitor gene family contains at least 10 members (Jofuku and Goldberg, 1989). Four of these members have been cloned and characterized (Jofuku and Goldberg, 1989, Song et al., 1993). Kti3 and KTib encode Ti^a and Ti^b respectively (Kim et al., 1985). Some of these genes are regulated temporally and spatially during embryogenesis (Jofuku and Goldberg, 1989).

Proteinase inhibitors have been proposed to function as storage proteins, regulators of endogenous proteinases, or factors that protect plants from insect attack. However, proteinase inhibitors are the major untinutritional substances for their too high content in soybean seed. Because soybean null lines exist that lack the Kunitz trypsin inhibitor, this protein is not essential for normal growth and development. The beans with and without the Kunitz trypsin inhibitor have the same yield, maturity date, lodging score, height, seed quality, seed weight, percent protein, and percent oil (Hymowitz, 1985). Therefore, selection of lines without SKTi that improves soybean nutritional quality is an important target in soybean breeding program.

Combining the use of RAPD and NILs provides a route for quickly identifying markers linked to a trait of interest. The linkage of a RAPD marker is usually identified by analyzing the F2 segregating population. Our result of RAPD analysis in six F5 lines and 40 seeds of the cultivar 92NK08 all carrying the *titi* gene are perfectly consistent to the result of enzymatic assay, indicating that the 560bp marker can be used in breeding program for soybean nutritional improvement. The result of analyzing F_2 population will be reported elsewhere.

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Screening for Resistance to *Phytophthora sojae*

Introduction

Phytophthora root and stem rot of soybean, incited by *Phytophthora sojae* M. J. Kaufmann & J. W. Gerdemann (syn. *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin), was first noted in the United State in 1948(Schmitthenner, 1985). Since that time this disease has been found in main soybean growing areas of the world, and was one of the most destructive diseases for soybean production. *P. sojae* is a highly variable pathogen. By 1995, 39 physiological races had been described by their virulence on a set of differential soybean varieties following seedling hypocotyl inoculation (Henry and Kirkpatrick, 1995).

In China, *P. sojae* was first isolated in Heilongjiang, Jilin and Beijing in 1989 (Shen and Su, 1990, Su and Shen, 1993). At present, phytophthora root rot of soybean is becoming an important problem in Heilongjiang province (Li and Ma 1996). Some isolates of *P. sojae* have been demonstrated that had different virulence (Zhou et al, 1995), race 1 has been identified (Zhu and Wang, 1998), but screening of commercial soybean cultivars and soybean germplasm for resistance to *P. sojae* has not occurred in China. This paper reports evaluation of main commercial soybean cultivars and some breeding lines from in Heilongjiang and Jilin resistance to race 1.

Materials and Methods

1. Inoculum and plants production

An isolate of race1, isolated from soybean cv Hefeng 25 in Jiamusi, Heilonjiang province, was cultured on petri plates containing semi-solid dilute V8 agar medium and incubated at 25under darkness. 8-day-old cultures were used for inoculation.

Commercial soybean cultivars and breeding lines for resistance evaluation were obtained from Institute of Crop Germplasm Resources, CAAS. 15 seeds of each cultivar or line were planted in 12-cm-diameter pot, pots were placed in a greenhouse at 22 to 27, Sunlight was supplemented with 1250 W (2505) high-pressure mercury lamps for 14 hours per day. The seedlings were thinned to ten uniform seedlings per pot immediately before inoculation.

2. Resistance evaluation

The hypocotyl inoculation technique using mycelium was used for screening of soybean resistance (Haas and Buzzell, 1976). 8-day-old cultures of *P. sojae* were macerated through a 50-ml syringe with No.9 needle, and loaded in a 5-ml syringe, a No. 7 needle was placed the end of the syringe. 10 10-day-old seedlings of each cultivar and line were inoculated. A 1cm vertical slit in the hypocotyl 0.5 cm below the cotyledonary node was made with the needlepoint, and a small quantity of inoculum was extruded into tbe slit. The seedlings were then placed in a mist room for 24 - 48 h at 18 - 25°C. Reactions of soybean seedlings were evaluated 4 to 5 days after inoculation. A cultivar or line was classed as susceptible if 70% or more of the seedling were killed and resistant if 30% or less of the seedlings were killed. Seedling mortality ranging from 30 - 70% was considered an intermediate reaction.

Results and Discussion

82 commercial soybean cultivars and 53 breeding lines from Heilongjiang and Jilin province were evaluated for resistance to race 1 (table 1). 16 of 82 commercial soybean cultivars were resistant, but resistance to race 1 was relatively common in soybean breeding lines, 50.94% of 53 lines were resistant. In 1989, Su and Shen (1993) isolated *P. sojae* for the first time in Heilongjiang, Jilin and Beijing, but since then no new reports of soybean phytophthora root rot disease in Jilin and Beijing have been published. The lack of resistant genes in commercial soybean cultivars maybe an important reason that phytophthora root rot is becoming a serious disease in Heilongjiang.

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Table 1. Reaction of commercial soybean cultivars and lines to race 1 of *P. sojae*

Cultivars	Source[a]	Reaction[b]	Lines	Source	Reaction
Heinong 37	HLJ	S	Longping 93-454	HLJ	S
Heinong 38	HLJ	S	Longping 88-1150	HLJ	S
Heinong 40	HLJ	S	Longping 90-055	HLJ	S
Dongnong 42	HLJ	S	Sui 91-41052	HLJ	R
Suinong 10	HLJ	R	Sui 93-355	HLJ	R
Suinong 11	HLJ	R	Sui 93-487	HLJ	R
Suinong 12	HLJ	S	Sui 93-368	HLJ	S
Suinong 13	HLJ	S	Bianjing	HLJ	R
Suinong 14	HLJ	S	D8904-2	JL	R

Cultivars	Source[a]	Reaction[b]	Lines	Source	Reaction
Kangxian 12	HLJ	R	Jinong 9016-11-3	JL	R
Kefeng 22	HLJ	S	Gongjiao 9076-89	JL	R
Nenfeng 15	HLJ	R	GY95-24	JL	R
Kennong 4	HLJ	R	D9202-1	JL	S
Hefeng 33	HLJ	S	Tongjiao 91-1591	JL	S
Hefeng 34	HLJ	R	Gongjiao 90136-25	JL	R
Hefeng 35	HLJ	S	Jinong 8821-19	JL	S
Hefeng 36	HLJ	S	Danjiao 91082-m-8	JL	R
Hefeng 37	HLJ	S	Changjiao B95-69	JL	R
Heihe 10	HLJ	S	Gongjiao 91116-1	JL	S
Heihe 11	HLJ	S	Gongjiao 91114-31	JL	S
Heihe 12	HLJ	S	Danjiao 8504-8-3	JL	S
Heihe 13	HLJ	S	Jiujiao 9193-14	JL	R
Heihe 14	HLJ	S	Changjiao 8715-9231	JL	R
Heihe 15	HLJ	S	Gongpei 8906-9-24	JL	R
Hongfeng 7	HLJ	S	Sijiao 8832-1	JL	R
Hongfeng 9	HLJ	I	Gongjiao 9223	JL	R
Hongfeng 10	HLJ	S	Changjiao B95-21	JL	S
Baofeng 2	HLJ	S	Jiujiao 9193-12	JL	I
Baofeng 3	HLJ	S	D91071	JL	I
Jiufeng 6	HLJ	S	Tongjiao 911571	JL	R
Jiufeng 7	HLJ	S	Gongjiao 9141-10-1	JL	S
Beifeng 13	HLJ	S	Tongjiao 91-1549	JL	S
Dongnong 9674	HLJ	S	Changjiao 8928-11161	JL	S
Juteqifenghuang	JL	I	Gongjiao 90136-1	JL	R
Wangzhongwang2	JL	R	Jinong 8714-13	JL	S
Wangzhongwang 1	JL	R	Changjiao B95-33	JL	R
Jifeng 2	JL	R	Jiujiao 91123-2	JL	R
Jilin 29	JL	S	Jiujiao 91102-9	JL	R
Jilin 30	JL	S	Gongjiao 91131-40	JL	S
Jilin 31	JL	S	Yuan 94001	JL	I
Jilin 32	JL	I	Jinong 8709-42283	JL	S
Jilin 33	JL	R	Jiujiao 9125-4	JL	R
Jilin 34	JL	R	Gongjiao 90208-114	JL	S
Jilin 35	JL	I	Gongjiao 9097B-1	JL	S
Jilin 36	JL	R	Tongjiao 90-397	JL	S
Jilin 37	JL	R	Jinong 8551-3322	JL	S
Jihuang 38	JL	S	90RD38-4	JL	R
Jihuang 50	JL	S	Jiujiao 8866-12	JL	S
Jihuang 72	JL	R	Jinong 8840-735	JL	R
Jihuang 85	JL	S	Jiujiao 9809-16	JL	R
Jihuang 132	JL	S	Sijiao 7815-31	JL	R
Jihei 10	JL	R	Changjiao B94-92	JL	R
Jihei 27	JL	R	Changjiao 93-4	JL	S
Jihei 53	JL	S			
Jihei 56	JL	S			
Jiqing 38	JL	S			
Jiqing 41	JL	S			
Jiqing 62	JL	S			
Jiqing 63	JL	S			
Jiqing 93	JL	S			
Jiqing 94	JL	S			
Jiqing 96	JL	S			
Jiqing 98	JL	S			
Jiqing 99	JL	S			
Jiqing 101	JL	S			
Jiqing 103	JL	S			
Jiqing 106	JL	S			
Jiqing 110	JL	S			
Jiqing 112	JL	S			
Jiqing 114	JL	S			
Jiqing 116	JL	S			
Jiqing 118	JL	S			
Jiqing 119	JL	S			
Jiqing 125	JL	S			
Jiqing 127	JL	S			
Jiqing 129	JL	S			
Jiqing 131	JL	S			
Jiqing 132	JL	S			
Jiqing 134	JL	S			
Jiqing 136	JL	S			
Jiqing 140	JL	S			
Jiqing 154	JL	S			

a: HLJ = Heilongjiang province; JL = Jilin province

b: S = Susceptible (70 or more plants kill); R = Resistant30% or less plants kill; I = Intermediate

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Soybean Performances in Xinjiang Autonomous Province of China — A Promising New Soybean-Producing Region

Introduction

Xinjiang Autonomous Province is located in Northwest China. It is a vast area with a small population. Soybean has been tried in production since 1950's, due to its low yield and poor seed quality, it is still limited to very small acreage. In order to solve this problem, we introduced various types of varieties from Northeast China, started our own breeding program in Shihezi City region. For Shihezi City, the latitude is 44°19'N, the longitude is 86°03'E, the elevation is 442.9m; the average temperature for July is 25.3°C, the highest for July is 32.2°C; the accumulated temperature (≥10°C) is 3478.1°C. In 1991, 1993, 1994, 1995 and 1996, numerous introduced lines and varieties and advanced lines (all are referred to as varieties hereafter) from our breeding programs were yield tested. The results are shown in Table 1.

Apparently, varieties with maturity <110 days were not competitive in yield. The average yield never reached 2700.0 kg/ha, it could be as low as 1241.3 kg/ha (as in 1995). Although the harvest indexes of this type of varieties were high, the plants were short, also with smaller seeds and less seeds per plant, their total biomasses were low. The varieties with maturity >140 days also performed poorly in yield. Usually, these cultivars could not mature normally before the frost comes, the seed quality was poor, the water content of seeds was high. Although these late varieties had high biomass, the harvest index was low, the 100-seed weight was light. Only in the year when frost was late, the late varieties could have a better yield. The varieties with maturity ranging from 111 to 140 days are the right type for Shihezi City region. In the first year experiment (1991), the yield for 111-120, 121-130, 131-140 day maturity groups were 2958.0 kg/ha, 2853.0 kg/ha and 2148.0 kg/ha, respectively. In the second year (1993), all were over 3373 kg/ha. In the last tree years, the average yield for all the three groups was over 4000 kg/ha. These results indicate that the best choice is the group of varieties with maturity of 125-135 days, these varieties could make full use of the heat, sunlight, and water resources of the growing season; the varieties usually have a plant height of 70-80cm, resistance to lodging, semideterminate growth habit, and oblong or small ovate leaflets. One-hundred seed weight of 18-22g is preferred.

Reducing the row width from 60cm to 33cm or 45cm greatly increased yield, the average yield increase was about 20-30%.

Northeast China is the major soybean-producing region, also with higher yield per hectare. The average yield in production is about 2250 kg/ha. But our experiments showed that better yield could be achieved in Shihezi, Xinjiang. In most experiments conducted in our program, the yield was higher than 3000 kg/ha, it was very easy to get a yield of 4000 kg/ha. Average yield of 4638.5 kg/ha with 14 varieties with 121-130 day maturity was achieved in 1994, similar result appeared again in 1996. Therefore, if right cultivars are used and suitable culture measures are practiced, Xinjiang could become another major soybean-producing region in China.

Table 1. Agronomic Performances of Soybean Varieties with different maturity

Year	Maturity Group (Days)	No. of Varieties	Maturit (Days)	Plant Height (cm)	Nodes per Plant	Pods per Plant	Seeds per Plant	Harvest Index	100-Seed Weight (g)	Yield (kg/ha)
1991	<100	3	99.3	42.1	9.7	19.3	45.6	.56	18.6	2583.0
	101-110	8	105.5	53.7	11.5	23.3	61.0	.55	19.0	2683.5
	111-120	12	115.3	75.8	14.3	34.8	83.8	.48	15.6	2958.0
	121-130	7	125.7	75.2	15.6	37.1	90.1	.44	16.4	2853.0
	131-140	2	133.5	77.3	14.5	29.1	59.1	.35	19.3	2148.0
1993	101-110	7	102.0	48.3	9.7	22.1	51.7	.56	19.1	2677.5
	111-120	7	114.0	63.5	10.6	22.9	55.1	.55	17.7	3373.5
	121-130	3	123.3	77.7	13.1	29.4	76.4	.52	17.2	3685.5
	131-140	7	136.3	70.3	13.0	22.1	53.5	.47	20.2	3459.0
	141-150	2	145.0	87.5	15.4	26.3	60.3	.44	19.6	3354.0
1994	101-110	1	106.0	46.1	12.8	24.9	66.0	.60	17.7	2367.0
	111-120	17	116.6	63.3	13.0	31.6	73.7	.56	20.7	4213.5
	121-130	14	123.6	77.4	14.6	32.3	79.5	.54	20.9	4638.5
	131-140	10	135.4	83.8	15.0	43.1	93.8	.49	22.9	4580.4
	141-150	29	143.6	119.6	20.2	46.6	90.9	.33	17.9	3147.0
1995	101-110	3	111.0	25.6	8.5	13.0	27.3	.60	20.8	1241.3
	121-130	15	127.3	58.8	13.2	30.5	73.1	.56	21.7	4203.0
	131-140	14	135.1	58.7	14.1	40.3	97.1	.55	23.7	4228.8
	141-150	3	144.3	141.2	23.2	57.7	135.0	.43	21.1	3978.3
	>150	3	161.7	80.8	15.6	56.0	115.0	.43	19.4	3442.2
1996	111-120	14	117.4	74.7	15.1	32.1	80.6	.49	18.6	4054.8
	121-130	11	126.0	77.1	14.7	27.4	61.0	.49	20.3	4261.2
	131-140	10	135.1	87.5	18.5	29.9	72.6	.51	21.3	4636.8
	141-150	3	141.7	108.1	16.8	28.9	46.0	.40	20.4	3936.0

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Studies on Resistance Mechanism of Soybeans to Leaf Feeders

Introduction

It was reported that in Nanjing, China, the major species of leaf feeders for soybeans were bean pyralid [*Lamprosema indicata* (Fabricius)], mugwort looper [*Ascotie Selenaria* (Schiffermuller et Denis)] and cotton worm [*Prodenia litura* (Fabricius)] and the population structure in the field varied from year to year, which were quite different from the situation in the US. The methods of evaluation and classification based on defoliation percentage with multiple observations were proposed and used as the indicator of resistance to leaf feeders. From 6724 accessions, 20 highly resistant and 12 highly susceptible ones were screened out by using the proposed criterion. The present paper deals with the mechanism of resistance of soybeans to the local leaf feeders in Nanjing, China.

Materials and Methods

1. Field test under natural insect population

Forty-six resistant and susceptible previously identified accessions were tested further in randomized blocks under natural leaf feeder population in 1993-1996. The data were obtained for defoliation percentage, 4-5 times each year.

2. Net room test under artificial infestation with cotton worm

The materials used were three highly resistant accessions, N3967, N3039, and N1178-2-2, and three highly susceptible ones, N21266, N119-1, and N20839, tested in randomized blocks in 1996 with two times of inoculation of young larvae of cotton worm, five times of observations of defoliation percentage. The larvae were picked up and collected in the field, and then were mixed up for infestation.

3. Field test for ovipositional preference

Three highly resistant accessions, N5454-3, N3967, and N2549-2 and three highly susceptible ones, N119-1, N1222, and N20839, were tested in the field with natural infestation of leaf feeders, in three latin squares. The numbers of eggs of mugwort looper, bean pyralid, cotton worm, and globular stink bug were inspected weekly.

4. Net room test for ovipositional preference under artifical infestation with cotton worm

The same set of materials in "2" were tested in net room infestated with adult moth of cotton worm. The numbers of eggs laid were counted.

5. Leaf-feeding test

The leaves of the same set of materials in "2" were used to feed the cotton worm larvae in three consecutive tests. The amount of leaf consumption, larval weight, larval mortality, pupal weight, pupal mortality, larval developmental period, and pupal developmental period were observed.

Results

1. Filed experiment under natural infestation

The major leaf-feeding insect species in the field during 1993-1996 were cotton worm, bean pyralid and mugwort looper. There existed significant differences among the tested 46 accessions in defoliation percentage. So did for accession year interaction. In Table 1, listed were the results of three highly resistant and three highly susceptible accessions which were used later in the test for antibiosis. It was obvious that there appeared significant differences in defoliation percentage between the two types of accessions in three years except 1995, which indicated the existence of accession year interaction.

2. Resistance to cotton worm under artificial infestation in net-room

Table 2 indicated the significant differences in defoliation percentage between the two sets of accessions, the resistant ones being of reduced defoliation and the susceptible ones being of increased defoliation.

3. Results from field ovipositional preference

Table 3 indicated that the overall trend was the resistant accessions having less eggs laid and the susceptible ones having more eggs laid by the four major species of leaf-feeders for soybeans.

4. Results from ovipositional preference in net room under artificial infestation with cotton worm

The numbers of pieces of eggs laid on resistant accessions were less than those on susceptible ones, but not significant due to the large error mean square. (Table 4).

5. Results from leaf-feeding test to show the antibiosis to cotton worm

Table 5 showed that the leaf consumption per single larva on resistant accessions was less than that on susceptible ones; the single larva weight on resistant accessions less than that on susceptible ones; the larval mortality on the former larger than that on the latter; the larval development period on the former longer than that on the latter; and the single pupal weight on the former less than that on the latter. There was no significant difference for pupal mortality and pupal development period between the resistant and susceptible types. It seemed that the antibiosis was mainly reflected on the growth and development of larvae, and its influence to pupae was not as significant as to larvae.

Conclusion

There exists the field resistance of soybeans to leaf-feeders, including bean pyralid, mugwort looper, cotton worm, and globular stink bug in Nanjing, China, which performs on reduced defoliation and oviposition amount, indicating the existence of antibiosis and non-preference.

The resistance mechanism of soybean to cotton worm performs on mainly antibiosis which appears on reduced foliage consumption, larval weight, pupal weight, and increased larval mortality and elongated larval development period, as well as on nonpreference, which appears on reduced oviposition amount.

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Table 1. Defoliation percentages of resistant vs. susceptible accessions under natural infestation in field.

Type	Accession	1993	1994	1995	1996
Resistant	N3967	18.71 b	30.55 b	25.90 ab	15.76 c
	N3039	21.48 b	29.16 bc	27.27 ab	17.44 c
	N1178-2-2	22.61 b	25.06 c	22.45 b	21.05 c
Susceptible	N21266	50.11 a	46.20 a	28.86 a	38.46 a
	N20839	51.37 a	45.43 a	31.17 a	37.73 a
	N119-1	36.47 a	47.78 a	29.06 a	28.75 b

Note: a, b, c denote significant at 0.05 level.

Table 2. ANOVA of defoliation percentage and significance between resistant and susceptible accessions.

Source of Defoliation	DF	MS	F	Resistant accession	Defoliation %	Susceptible accession	Variation %
Accession	5	232.72	29.79**	N3967	28.4 B	N21266	41.4 A
Block	5	12.20	1.56	N3039	29.0 B	N20839	39.4 A
Error	25	7.81		N1178-2-2	28.9 B	N119-1	39.2 A

Note: ** as well as A and B denote significant at 0.01 level.

Table 3. Numbers of eggs per plant laid by the four major species of leaf-feeders for soybeans.

Type	Accession	Mugwort looper	Bean pyralid	Cotton worm	Globular stink bug
Resistant	N5154-3	0.36 c	0.78 bc	0.39 d	2.28 d
	N3967	0.31 cd	0.58 c	0.19 e	2.03 d
	N2549-2	0.17 d	0.47 c	0.36 d	3.78 d
Susceptible	N119-1	0.33 c	0.94 b	0.67 c	7.94 c
	N122	0.50 b	0.58 c	0.89 b	16.97 a
	N20839	1.08 a	1.44 a	1.50 a	12.81 b

Note: a, b, c, d, e denote significant at 0.05 level. Those for cotton worm are number of pieces of eggs per plant.

Table 4. ANOVA of ovipositional preference of cotton worm and comparisons between resistant and susceptible accessions

Source of variation	DF	MS	F	Resistant accession	No. pieces of eggs per plant	Susceptible accession	No. pieces of eggs per plant
Accession	5	1.83	0.89	N3967	3.0	N21266	4.5
Block	5	3.16	1.54	N3039	3.8	N20839	4.0
Error	25	2.05		N1178-2-2	3.5	N119-1	4.3

Table 5. Response of growth and development of cotton worm larvae to resistant and susceptible accessions.

Type	Accession	Leaf consumption per plant (g)	Single larva weight (g)	Larval mortality (%)	Period of larval development (d)	Single pupa weight (g)	Pupa mortality (%)	Period of pupa development (d)
Resistant	N3967	1.847 b	0.355 c	35.0 a	16.0 a	0.174 c	7.5	7.6
	N3039	1.848 b	0.341 cd	33.3 a	16.4 a	0.166 c	7.3	7.5
	N1178-1-2	1.921 b	0.324 d	40.0 a	16.1 a	0.154 d	6.8	7.9
Susceptible	N21266	2.427 a	0.485 b	21.7 bc	13.7 b	0.191 b	5.3	7.7
	N20839	2.414 a	0.492 b	22.5 b	13.1 b	0.199 ab	6.3	7.8
	N119-1	2.564 a	0.527 a	14.2 c	14.0 b	0.204 a	6.8	7.6

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The Comparison of Resistance Gene Analogs from Kefeng No.1 and PI96983

Introduction

Of late years, genes for resistance to pathogens(R genes) have been cloned from higher plants. Sequence comparisons between these genes such as RPS2 and RPM1 in arabidopsis, N in tobacco, Xa21 in rice, L6 in flax, Cf-2 and Cf-9 in tomato revealed structural similarities; all of them contain leucine-rich repeats(LRR) and motifs for a conserved nucleotide binding site(NBS) are also found in RPS2, RPM1, N and L6 coding region, although these genes confer resistance to viral, bacterial and fungal pathogens respectively.

The high degree of sequence similarity between R genes of different plants led us to consider that the conserved motifs of R gene may also present in related genes in other plants. Several sets of degenerate oligonucleotide primers have been designed to amplify resistance gene analogs(RGA) by PCR reaction. Some of these RGA sequences have been mapped as genetic markers tightly linked to known resistance genes, and these amplification products would be suitable as probes for the isolation of genes resistant to different types of pathogens.

In present study, we used degenerate oligonucleotide primers to amplify RGAs from two SMV-resistance soybean varieties, Kefeng No.1 (from China) and PI96983 (from USA). Our objectives were to provide some information for the exploitation of rare SMV-resistance resources and to map and isolate SMV-resistance genes.

Materials and Methods

Plant materials and DNA extraction

Two SMV-resistant varieties Kefeng No.1(from China), PI96983(from USA) and a SMV-susceptible cultivar Nannong 1138-2(from China) were adopted as the DNA source for PCR amplification. Their young leaves were collected and total DNA was extracted using the CTAB method.

Design of degenerate primers and amplification conditions

Two amino acid sequences with 100% homology in N and RPS2 were used to design degenerate primers (Table 1). Primer 1 was designed from the conserved p-loop motif (GGVGKTT) of the NBS; primer 2 was designed from the second sequence (GLPLAL) about 160 amino acids (500bp) further downstream, this sequence is probably in a transmembrane region. Both motifs are located in the N-terminal half of the putative proteins upstream of the LRR region.

Table 1. Sequences of the two degenerate primers

Primers	Sequences 5' 3'
primer 1	TCCGGTGGGGTTGGGAAGACAACG
primer 2	TCCCAACGCTAGTGGCAATCC

Amplification reaction was carried out in 50l of reaction volume containing 10 mM Tris-HCL, 50mM KCL, 100M dNTPs, 0.1M each primer, 1 unit Taq DNA polymerase, 2.0 mM Mg2+ and 100ng template DNA. Reactions were performed in a Perkin-Elmer Cetus DNA-thermocycler 9600. Amplification conditions were 1 cycle of 93, 2 minutes; 35 cycles of 93, 50 seconds, 53, 50 seconds, 72, 1.5 minutes; 1 cycle of 72, 10 minutes.

Cloning and Sequencing PCR products

The amplification products were resolved by gel electrophoresis, fragments were excised and purified by the 'Gene Clean' kit, and blunt-end ligated into the pGEM-T easy vector from Promega. A portion of the cloned PCR products were digested with EcoR I and the fragments were identified by size comparison with the original PCR products. Double-stranded sequencing of the cloned fragments was done by the dideoxy-chain termination method with 373A DNA Sequencer.

Results and Discussion

Amplification products

As we have known that Kefeng No.1 carrys SMV-resistance gene Rsa and it is almost resistant to all of SMV strains such as Sa, Sc, Sg, Sh, N1, N2, N3; PI96983 contains Rsv and is resistant to Sc, Sg, G1, G2, G3, G4, G5, G6; Nannong 1138-2 is susceptible to Sa, Sc, Sg, Sh, N1, N2, N3. When primer 1 was used singly to amplify total DNA from Kefeng No.1, four fragments of approximately 3kb, 2kb, 600bp and 300bp were obtained, while when primer 2 was singly used, no product was obtained. However both of primer 1 and primer 2 were used in reaction two fragments of about 2kb and 500bp were appeared from Kefeng No.1, PI96983 and Nannong 1138-2 respectively. A slight polymorphism was found between two resistant varieties and the susceptible variety Nannong 1138-2 in the band of 500bp. These fragments were similar to the conserved motifs of N and RPS2 in size. Therefore, the fragments of about 500bp were retrieved and named accordingly as Fk (from Kefeng No.1), Fp (from PI96983) and Fn (from Nannong 1138-2).

Cloning and sequencing PCR products

The fragments mentioned above were cloned and six clones with Fk, 4 clones with Fp and 3 clones with Fn were obtained. These cloned products were named as Fk1 - 6, Fp1 - 4, Fn1 - 3. Restriction enzyme analysis with EcoR I and sequencing results showed that amplification products Fp and Fn consisted of homogeneous DNA sequences, that is, Fp1 - 4 have a identical sequence of 489bp, the sequence was called Sp hereafter; Fn1 - 3 have a consistent sequence of 417bp, the sequence was nominated as Sn. While the amplification products Fk contained a mixture of two different DNA fragments, that is, Fk1, Fk3, Fk4, F5 and F6 have a same sequence of 489bp, Fk2 has a sequence of 417bp, these two sequences were named as Sk1 and Sk2.

Sequence comparison results showed that Sk1 had a similarity above 99% with Sp, only two bases in 489bp were different from Sp (Table 2). While Sk2 had a homology of nearly 100% with Sn, only one base in 417bp was not the same as in Sn (Table 3). Sk1 and Sk2 were quite different, except the two ends including primers, only shared several bases from 5' end to 3'end at intervals.

When Sk1 and Sp were used as probes, Southern blotting showed that there was only one band therefore this fragment was a single copy DNA of soybean. Because Sk1 and Sp both originated from SMV-resistance varieties (Kefeng No.1 and PI96983 respectively), we are trying to use them as molecular markers to map SMV-resistance genes. While Sk2 and Sn were generated from Chinese varieties (Kefeng No.1 and Nannong 1138-2), they would be useful in researches concerning soybean evolution.

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Table 2. The comparison of Sk1 from Kefeng No.1 with Sp from PI96983

```
        10        20        30        40        50        60
Sk1 TCCGGTGGGG TTGGGAAGAC AACGAAAACA TTGAACACTA AGCTTGGGTA TGGATACAAG
Sp  TCCGGTGGGG TTGGGAAGAC AACG..... ..........  ..........  ..........
        70        80        90       100       110       120
Sk1 GCGCGCGTCA GGGAATGTGT CATGGGGTCA AATTAGGGAT CGGATCTTTG GCTTTTAGGG
Sp  ..........  ..........  ..........  ..........  ..........  ..........
       130       140       150       160       170       180
Sk1 TCGATACTGT GTTATTTTAT TTGGAGAGAA TTGATTTGAT ATTGGGAATT GCTTGGTTGG
Sp  ..........  ..........  ..........  ..........  ..........  ..........
       190       200       210       220       230       240
Sk1 CACCATTGGG GAAGATGATG ATTGATTGGG GGGAATTGGT TATGCTGATA AAAGTAGATG
Sp  ..........  ..........  ..........  ..........  ..........  ..........
       250       260       270       280       290       300
Sk1 ATAAGTGGGT CATAATACAA GGGGAAGGGA CACGTGTGTG AGCAAACAGC TTTTCAAACG
Sp  ..........  ..........  ..........  ..........  ..........  ..........
       310       320       330       340       350       360
Sk1 TTGTTGGGAC GATGCAAAAG TATGGTTGAG GGGTTGTTGT GGTCGACAGA AGTGACCTTG
Sp  ..........  .........A ..........  .....C.....  ..........
       370       380       390       400       410       420
Sk1 CCCAATCCCA AGAAGGAAGA GAAGTTGTCT AGTTCAATCA TGGCAACCCA GGAGCAGGAA
Sp  ..........  ..........  ..........  ..........  ..........  ..........
       430       440       450       460       470       480
Sk1 TTGAAAGCCC TGTTAGGAAG TTTCAGCAAG GTGTTCAGAG AACCCCATGG ATTGCCACTA
Sp  ..........  ..........  ..........  .........GG ATTGCCACTA          489
Sk1 GCGTTGGGA
Sp  GCGTTGGGA
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Table 3. The comparison of Sk2 from Kefeng No.1 with Sn from Nannong 1138-2

```
        10        20        30        40        50        60
Sk2 TCCGGTGGGG TTGGGAAGAC AACGAAATAA GGATAGGTCC AATTTATAAA ATACAGGTGC
Sn  TCCGGTGGGG TTGGGAAGAC AACG..... ..........  ..........  ..........
        70        80        90       100       110       120
Sk2 ACCAATCAAA CAAGTTTCAC ACAAACAATC AAACAGAAAA GTCACAAAAA CAATCCCCCG
Sn  ..........  ..........  ..........  ..........  ..........  ..........
       130       140       150       160       170       180
Sk2 AGCCTTGCCT CCTAAGTCCT AACGAAAAAG AGGAGAAAGG AAAGGCCCAT TGAACGTTTG
Sn  ..........  ..........  ..........  ..........  .......C..
       190       200       210       220       230       240
Sk2 CAATTTTTTA CAGCATAATG CAGCCGTTTA AGTCAGCAAT TTTGACAGCC CAAATTCTTG
Sn  ..........  ..........  ..........  ..........  ..........  ..........
       250       260       270       280       290       300
Sk2 CAATTAAGTA CCTGAACTCG ACGAACGGCT CCAACTCTGC ACGTGTCTCC GTTCAAACAA
Sn  ..........  ..........  ..........  ..........  ..........  ..........
       310       320       330       340       350       360
Sk2 CTCCAACTAC GGTTACGCCG TCGAGATTCA GAGAGGGAGT GGGCCGGCGA GATTGAGAAA
Sn  ..........  ..........  ..........  ..........  ..........  ..........
       370       380       390       400       410   417
Sk2 GGGGCCAGGG TTGTGCGCCG GCGAGGCTTA GAAACGGGAT TGCCACTAGC GTTGGGA
Sn  ..........  ..........  ..........  ......GGAT TGCCACTAGC GTTGGGA
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Varietal Differences of Sprout Characters of Soybeans

Introduction

Soybean sprout is a popular food in China, and an excellent source of protein, minerals and vitamins. There were compliments about the food and medicinal values of soybean sprouts in the ancient Chinese book "Shen Nong Cao Jing" written in the first century. Most people eat yellow soybean sprouts, some people in central China also eat black soybean sprouts. Usually soybean sprouts are cooked before serving. They could be cooked with other vegetables or meat. Soybean sprout soups are also very delicious. In the United states, with increasing health concern resulted from consuming too much meat, high protein soybean foods are getting the attention of many people, suggestions were made using soybean sprouts as a green vegetable parboiled in salads or soups, or in stir-fried, sautéed, or baked dishes (Indiana Soybean development Council, 1997). Soybean sprouts in the market are produced by farmer household or urban household, these household just use any soybean seeds they can get to produce the sprouts. Very little information is available for the sprout characters of soybean varieties. People usually believe that small-seed varieties are better for sprout use, and have higher sprout yield. The objectives of this investigation are (1) to study the effect of temperature on sprout characters of soybeans; (2) to investigate the varietal differences of sprout characters. The experiment is not yet finished, preliminary results are presented here.

Materials and Methods

Seven varieties were used in the study. All of them were collected from the Soybean Research Institute, Northeast Agricultural University, Harbin, Heilongjiang Province. Seed coat color and seed size were considered when choosing the experimental materials (Table 1). The temperature treatments were 20°C, 25°C, 30°C, and 35°C. Five out of 7 varieties did not germinate well under 35°C, therefore, for the most part only 3 temperature treatments were presented. Characters studied were 100-seed weight (g), sprout length (cm), fresh weight (g)/100 sprouts, dry weight(g)/100 sprouts, fresh sprout yield (g)/kg seeds, and dry sprout yield(g)/kg seeds. Sprouts were produced in a growth chamber with 2 replicates, washing twice a day. The occurrence of literal roots is the criterion that sprouts are ready for use. The data were analyzed using Excel 5.0.

Results and Discussion

There were obvious differences for all characters studied among the varieties investigated (Table 1). For sprout length, the averages varied from 11.40cm of "DN690" to 7.60cm of "DN693" at 20°C, and from 10.35cm of "Heidou" to 3.80cm of "DN95-498" at 25°C. Black seed coat variety "Heidou" had long sprouts on average over the temperature treatments. Seed size did not have obvious relationship with sprout length. Fresh weight/100 sprouts and dry weight/100 sprouts were strongly positively related with seed size. Seed coat color apparently was not a factor here. Fresh sprout yield/kg seeds are very important for sprout sellers. There were great variations for this character among varieties, "Heidou", "DN92-19", and "DN690" had higher fresh sprout yield/kg seeds, seed size did not affect fresh sprout yield in this experiment. This is contradictory to the common view that small seed varieties have better sprout yield. There were variations for dry sprout yield/kg seeds among the varieties tested, generally small seed varieties had lower dry sprout yield/kg seeds.

Temperature is very important for producing soybean sprouts. Soybean seeds can only germinate and grow well at certain temperature. Different varieties may have different optimum temperature, in which maximum sprout yield and quality could be achieved. The data (Table 1) showed that soybean varieties responded differently to temperature in regard to sprout length, fresh weight/100 sprouts, dry weight/100 sprouts, fresh sprout yield/kg seeds, and dry sprout yield/kg seeds. For all 7 varieties, 20°C treatment produced the longest sprouts. "Heidou" still produced very long sprouts at 25°C (10.35cm, almost equal to the length 10.50cm at 20°C). All other varieties produced significantly shorter sprouts when temperature increased. Fresh weight/100 sprouts decreased with the increase of temperature in all varieties except "DN42", which had fresh weight/100 sprouts 80.07g, 71.30g and 74.57g respectively at temperature treatments 20°C, 25°C and 30°C. Apparently, 20°C is the best temperature for soybean sprout production. Dry weight/100 sprouts had the similar trends as fresh weight/100 sprouts in the aspect of varietal responses to temperature. All the varieties had the highest fresh sprout yield /kg seeds at 20°C; with the increase of the temperature, fresh sprout yield decreased significantly. "DN690" produced 4954.92g fresh sprouts/kg seeds at 20°C, only 3262.16g fresh sprouts/kg seeds at 30°C. Dry sprout yield/kg seeds responded to temperature the similar way as fresh sprout yield/kg seeds did. These results indicate that 20°C is the right temperature for soybean sprout production in general. This temperature is lower than the temperature recommended by Yang et al. (1989). The possible explanation is that the varieties used in this study were from Harbin, Northeast China, where soybeans were grown and germinated in cold spring field conditions, varieties were adapted to lower germination temperature.

In conclusion, there are significant variations of sprout characters among soybean varieties. Black seed coat varieties are good for sprout production if you do not mind the black color. Small seed varieties do not necessarily produce higher sprout yield. If no preference for single sprout size, large or small seed

varieties do not make any difference for producing sprouts, either type can produce long sprouts and can get high sprout yield. Different varieties respond differently to temperature in producing sprouts, generally 20°C is a better temperature for sprout production in Heilongjiang Province.

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Table 1. Sprout Characters of Soybean Varieties

	Heidou	DN42	DN92-19	Hefeng35	DN690	DN693	DN95-498	Average
Seed coat color	Black	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	
100-seed weight (g)	17.27	22.75	14.38	18.59	8.43	9.20	9.07	
				20°C				
Sprout length (cm)	10.50	7.85	11.25	7.80	11.40	7.60	7.90	9.19
Fresh weight (g)/100 sprouts	72.49	81.07	62.80	73.18	41.77	36.07	33.37	57.25
Dry weight (g)/100 sprouts	14.19	18.92	11.66	14.92	6.14	6.78	6.95	11.36
Fresh sprout yield (g)/kg seeds	4197.45	3563.52	4366.83	3936.26	4954.92	3920.11	3678.61	4088.24
Dry sprout yield (g)/kg seeds	821.37	831.43	810.85	802.58	727.76	736.96	766.26	785.31
				25°C				
Sprout length (cm)	10.35	5.50	6.90	5.15	8.45	5.90	3.80	6.58
Fresh weight (g)/100 sprouts	64.74	71.30	54.94	60.30	31.93	29.74	28.76	48.81
Dry weight (g)/100 sprouts	13.08	17.38	11.36	14.16	6.05	6.33	7.08	10.77
Fresh sprout yield (g)/kg seeds	3748.41	3133.85	3820.58	3243.68	3787.66	3232.07	3170.89	3448.16
Dry sprout yield (g)/kg seeds	757.09	763.74	789.99	761.43	717.08	687.50	780.04	750.98
				30°C				
Sprout length (cm)	8.20	7.75	8.10	4.90	5.10	5.70	4.10	6.26
Fresh weight (g)/100 sprouts	61.27	74.57	50.73	60.15	27.50	27.23	26.09	46.79
Dry weight (g)/100 sprouts	12.90	18.07	11.06	14.04	6.21	6.21	6.43	10.70
Fresh sprout yield (g)/kg seeds	3547.48	3277.80	3527.82	3235.34	3262.16	2959.78	2875.96	3240.91
Dry sprout yield (g)/kg seeds	746.67	794.29	768.78	754.98	736.65	675.00	708.38	740.68

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Biometrical Analysis for Grain Yield of Soybean Genotypes I Maturity Group of the Osijek Agricultural Institute – Croatia

Introduction

The soybean Glycine max (L) Merrill is relatively new crop in Croatia (28 years are growing in continuity). The main growing area is in the eastern part of country where are the best agroecological conditions for this kind of production. The research work on the soybean includes the breeding as well as the developing in that area at the Osijek Agricultural Institute. A full scale of soybean hybridisation programme was initiated at the Osijek Agricultural Institute in 1970 so far. Twenty-four soybeans recognised cultivars have been developed by hybridisation of selected parents. Soybean's breeding programme developed in Institute has enormous influence on the soybean production development and stability in the whole country, notably in the East Croatia. The main objective in the breeding of soybean is the permanent development of higher yielding cultivars (above 5 t/ha) in the frame of 00 to II maturity groups (0 and I maturity groups in focus). Besides, the high genetic yield potential of the new cultivars ought to have other agronomic traits satisfactory. The present work is organised only according to the conventional classic work methods and outlines valid for self-pollinated crops, such as soybean crop. The new biotechnological methods as the auxiliary methods to the classic are not introduced yet. In this paper, it will be given a part of results of our work on the soybean breeding for yield into genotypes of I maturity group with emphasis on heritability, genetic gain and grain yield stability. The results obtained should give the information about soybean genetic improvement for grain yield in soybean breeding program at the Osijek Agricultural Institute.

Material and methods

The microtrials with 29 soybean genotypes I maturity group were conducted at Osijek during the growth seasons 1993-1997. The genotypes selected for study were 26 lines derived from F_4-F_6 generation as promising lines into breeding programme of Institute and 3 cultivars (Tisa, Lika, Drina), also created in Institute, which are standard cultivars in large-scale soybean production last ten years in our country. Selected genotypes were grown simultaneously in a randomised complete block design with 3 replicates. The basic plot was 10m^2. Conventional agronomic measures for soybean were applied. Grain yield was weighed on each plot, after harvest, and converted to tone/ha with 13% moisture.

Biometrical analysis for grain yield was done by following parameters:

- mean values of grain yield for each investigated genotype;
- wide-sense heritability (%) was calculated from the analysis of variance, as the ratio of genetic variance to total phenotypic variance according to method by Singh et al (1993), where is:

$H=V_G/V_P$
$V_G=(MS_k-MS_I)/(nxg)$ MS-mean square n-number of replicates
$V_P=V_G+V_I+V_E$ K-cultivar g-number of years
$V_I=(MS_I-MS_E)/n$ E-environment
$V_E=MS_E$ V-variance

- achieved genetic gain was estimated after method by Allard (1960);
- relative genetic gain (%) was calculated as the ratio between genetic gain and mean value of grain yield;
- yield stability of studied genotype was described by: regression of mean yield on an environmental index (Finlay and Wilkinson, 1963) and portion of genotype x environment variance due to the contribution of each genotype to total variance of genotype x environment interaction (Plaisted and Peterson, 1959).

Results and discussion

Grain yield is the most important character in soybean. World-wide soybean breeders view yield as the main objective of their plant breeding programmes (Fehr, 1987; Ferraz de Toledo et al, 1994; Palmer et al., 1996; Vratari} et al., 1997). The results of the our research showed high variation among tested genotypes in grain yield. Grain yield of lines varied among 3.05 and 5.40 t/ha (mean 4.38 t/ha) and among 3.05 and 4.60 t/ha (mean 3.99 t/ha) for cultivars depended on year. (Tab. 1).

According to the average coefficient of regression (b=0.951), tested lines showed high yield stability (Tab. 2)

Heritability estimate for grain yield, based on a set of lines, varied from 29.96% to 36.08% (mean 34.54%). (Tab. 2). Relatively high yield heritability indicated that analysed lines can be used as parental material for improvement grain yield genetic potential. These results correspond with those described by Burton, 1987.

Our study indicated that soybean yield has increased by 0.0449 t/ha/year or 0.8%/year due to improved cultivars (Tab. 2). Similar results were reported by Voldeng et al, 1997.

According to the estimated parameters of stability, tested genotypes were differed in the stability. (Tab. 3, 4). Genotypes with higher than average grain yield and lower value then average interaction genotype x year can be considered as adaptable ones. The most stable genotypes, among 29 tested genotypes, were: L-2693, L-493, L-1093, L-393, L-7089, L-22290, L-4690, L-3493, L-1493, L-10692, L-37490 and Tisa.

Conclusion

Generally, the obtained results indicate progress in soybean breeding for grain yield at Institute and the possibility to create new cultivars of soybean with higher genetic potential for grain yield.

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Table 1. Average grain yield (t/ha) of investigated soybean genotypes, 1993-1997, Osijek.

Year	Grain yield				$LSD_{genotype}$	
	Rang		Average			
	Lines	Cultivars	Lines	Cultivars	0.05	0.01
1993	3.75-5.05	3.05-4.20	4.63	3.62	0.298	0.345
1994	4.30-5.60	3.70-4.60	5.09	4.10	0.311	0.367
1995	3.05-4.85	3.50-4.10	4.11	3.72	0.307	0.355
1996	3.00-4.42	3.54-4.30	3.84	3.83	0.319	0.403
1997	3.28-5.00	3.25-4.55	4.31	3.69	0.310	0.400
Average	3.44-4.90	3.44-4.35	4.39	3.79	0.324	0.427
LSD_{year}					0.135	0.177
LSD_{gxy}					0.628	0.814

Table 2. Parameters of the genetic improvement estimation on grain yield for investigated soybean lines.

Year	Average (t/ha)	Coefficient of regression	Heritability (%)	Genetic gain (t/ha)	Relative genetic gain (%)
1993	4.63	1.005	36.08	0.0432	
1994	5.09	1.096	35.99	0.0444	
1995	4.11	0.904	30.32	0.0427	
1996	3.84	0.822	27.96	0.0418	
1997	4.31	0.929	33.14	0.0448	
Average	4.396	0.951	34.54	0.0449	0.797

Table 3. ANOVA for grain yield of investigated soybean genotypes.

Source of variability	Df (n-1)	MS
Genotype (G)	28	609764
Year (Y)	4	15967777
Interaction(GxY)	112	335119
Error	288	23448
S^2_{GxY}		103890

Table 4. Average grain yield (t/ha) and parameters of stability for investigated soybean genotypes I maturity group, 1993-1997, Osijek.

Num.	Genotype	Grain yield (t/ha)	Parameters of stability			
			S^2_{GXY}		B	
1.	L-2693	4.90	97326	*	0.125	*
2.	L-493	4.74	102753	*	0.570	*
3.	L-1093	4.72	100244	*	0.284	*
4.	L-393	4.70	102560	*	0.416	*
5.	L-7089	4.69	89111	*	0.056	*
6.	L-22290	4.59	103418	*	0.318	*
7.	L-7093	4.58	104499		1.021	
8.	L-4690	4.57	102444	*****	0.910	*
9.	L-3493	4.57	103246		0.746	*
10.	L-1493	4.54	103281		0.765	*
11.	L-10692	4.50	101119		0.672	*
12.	L-37490	4.50	102803		0.771	*
13.	L-2393	4.49	105027		1.101	
14.	L-10990	4.49	104977		1.066	
15.	L-3390	4.48	106058	*	1.053	
16.	L-4993	4.48	104161		1.312	
17.	L-1693	4.44	105218		1.504	
18.	L-2593	4.39	106661		1.267	
19.	TISA	4.35	101720		0.932	*
20.	L-2090	4.26	105336		1.695	
21.	L-2193	4.25	104534		1.345	
22.	L-6090	4.22	103008		1.089	
23.	L-993	4.14	104772		1.257	
24.	L-5290	3.98	103619		1.077	
25.	L-20293	3.77	118672		1.768	
26.	L-1793	3.73	103910		0.988	
27.	DRINA	3.58	104362		1.167	
28.	L-20690	3.50	110454		1.542	
29	LIKA	3.44	107538		1.430	
Average		4.33	103890			

Legend:
S^2_{GxY}= portion of genotype x environment variance due to the contribution of each genotype to total variance of genotype x environment interaction
b = coefficient of regression
* = stable genotypes

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Study of Soybean Genome Diversity by Molecular Genetic Methods

Introduction

During last years, for studies of genetic diversity between soybean cultivars located in Mendeleum electrophoretical isozyme spectra have been used (Kadlec et al. 1994a, 1994b). Nowadays we have been started to solve the problem by methods of molecular genetics.

We used methods for isolating of genomic DNA from leaves, optimal for our conditions. Thus, first orientation studies of genetic diversity in rDNA areas of soybean genome have been obtained and a potential suitability of the RAPD technique as a tool for analyzing polymorphism of *Glycine max.* /L./ Merrill has been tested.

Material and methods

A. Two common methodologies (Dellaporta 1983 and Saghai-Maroof 1984) were used to isolate DNA from leaves. They differ in the way of separating the remains of cell walls after lytic dissociation.

B. In studies for measuring genetic diversity in soybean genome rDNA areas two hybrid cultivars bearing a part of *G. Soja* /L./ Sieb. & Zucc. were also included to increase the probability of obtaining polymorphous bands. The other cultivars were *G.max* /L./ Merrill.

Approximately 6µg of high-molecular genomic DNA isolated from leaves by the Dellaporta method were cleaved by five restriction endonucleases: Bam HI, Bgl II, Eco RI, Hind III, Bst NI. After agarose electrophoresis of restricted DNA and Southern blotting the membrane was hybridized with a radioactively marked probe isolated as Eco RI fragment of 25S rDNA area of potato genome (2.5 kb). Although the given area is rather conservative within the framework of eucaryotic organisms, some studies described polymorphism of the given area (Appels 1982, Kovarik et al. 1996, Borisjuk et al. 1986).

C. In addition to cultivars of *Glycine max.* genus two hybrids *G. max* x *G.soja* were included for testing the suitability of RAPD for observing genetic diversity. High-molecular genomic DNA isolated by the Dellaporta method was used as a template.

Oligonucleotides (10=mer) were used as primers:

a) commercial Operon primers randomly chosen

b) nucleotides prepared on the automatic nucleotide synthesizer, the sequences of which had been already proven as suitable for soybeans by other authors (Doldi et al. 1997).

The composition of the reaction mixture for RAPD amplification with the Operon primers was following:

- 50 ng of DNA as a template, 0.6 µM of primers, 1x reaction buffer, 1.5 mM of $MgCl_2$, 200µM of dNTP, and 0.5 U of Taq polymerase (all Promega) in a total volume of 25 µl.

The amplification yields were generally lower when synthesized primers were used and consequently the composition of the reaction mixture was modified:

- 100 ng of genomic DNA Soya as a template, 0.6 µM of a random primer, 1x reaction buffer, 1.5 mM of $MgCl_2$, 200 µM of dNTP, and 0.75 U of Taq polymerase (Promega) in a total volume 25 µl.

Thermocycler (Techne=Progene) was programmed in the following way:

- 1 minute at 94°C, followed by 40 cycles of 1 min at 94°C, 45 sec at 36°C, and 30 sec at 72° C, and a final cycle of 5 min at 72 °C.
- After amplification the samples were loaded on 1.5 agarose gel. The gels were colored by ethidium bromide and photographed by the Polaroid camera.

Results and discussion

The basic step of molecular genetic methods is the isolation of DNA with a high-quality. In RFLP the qualitative demands stem from the high-molecularity of DNA. Generally, the demand on preserving the sensitivity of isolated DNA to usually used enzymes (restrictive endonuclease, Taq polymarase, ligases, etc.) applies.

Recently, two methodologies or their modifications are frequently applied to isolate DNA from leaves. They differ in the way of separating the remains of cell walls after lytic degradation of a cell (Saghai-Maroof et al. 1984 – use the extraction into chloroform octanol mixture; Dellaporta et al. 1983 – use the detergent qualities of SDS). According to our experience the Dellaporta modification is more suitable unlike to Saghai-Maroof modification. The typical fibrillation of raw DNA occurred almost for all isolations. Moreover, higher mobility with the control electrophoreses of isolated DNA, indicating a spontaneous degradation, occurred less frequently with the Dellaporta modification. All these results have been achieved with lower time and centrifugation acceleration (g) demands, which indicates an easier separation of aqueous phase containing DNA.

The suitability of isolated samples was checked in the following RFLP study observing the polymorphism in rDNA area of soybean genome. The probe isolated from 25S-rDNA area of a potato genome was used. The autoradiogram after the hybridization of the transferred DNA (5 restriction enzymes applied) with this probe showed no observable polymorphism within the frame of the cultivars under observation. As for the richness of spectra, most hybridization bands per cultivar were

observed in the case of cleavage by BamHI enzyme (4 strong + 2 weak bands), 5 bands are observable in the case of cleavage by BstNI, and 4 bands with the cleavage by Bg1 II. Lowest bands, witnessing the hybridization, were shown in the audioradiogram after the cleavage by Eco RI, where 1 strong and 1 weak band can be seen for each cultivar. No hybridization was observed with the cleavage by Hind III.

As for our next RFLP applications, there is a general problem with the availability of suitable probes. If we are unable from the beginning to create our own genomic or cDNA libraries of a particular plant, it is possible to use the available probes isolated from more or less universal genome areas of other organisms. On the other hand the probability to get polymorphism usually reduced this way.

Amplification methods are promising tool to study the diversity of a plant genome. Methods, the most frequently cited for soybeans, are RAPD and SSR studies. Observing only stronger bands for particular primer they reduce the known problem with reproducibility. The following facts were noticeable:

1) Amplification of no product occurred with one of three randomly chosen Operon primers. In the case of OPI 07 primer a single considerable band was detected with every observed cultivar, always at the same distance from the start. The results of OPD 05 amplification revealed polymorphism – so the cultivars can be divided into two groups:
   a) cultivars providing no amplification product,
   b) cultivars providing one considerable band, always at the same distance from the start.
2) After amplification with the synthesized primers it was necessary to increase the amplification yields by modifying the composition of the reaction mixture (increasing the amount of template DNA and Taq polymerase in comparison to the reaction mixture for commercial primers). Amplification of three considerable bands for every cultivar occurred with 5' GGT GAC GCA G primer, but their layout has no different bands. Rather complicated spectrum was generated with 5' AAD AAC CCT C primer, in which two considerable places of variability could be found within the framework of the observed cultivar.

   With 5' TGC TCT GCC C primer the variability among the strongest amplified bands were observed from the amplification spectrum.

So, as a starting point, we have applied the primers that had been positively evaluated in literature. With them we have obtained rather more complicated spectra (which can be accounted to the modification of the reaction mixture), but especially a higher level of occurrence of polymorphous bands. This process does not necessarily mean the success, but a certain advantage with comparison to the application of randomly chosen primers is noticeable.

Conclusion

In Mendeleum's soybean breeding program the application of molecular genetic methods has been used. A suitable methods of isolating genomic DNA for both RFLP and amplification methods have been chosen. The suitability of isolated DNA has been tested by RFLP study concerning the study of variability in rDNA genome area. To increase the probability of getting polymorphism, hybrids of *G.max x G.soja* were included in the experiment. In spite of this, no variability between cultivars was detected.

For further development of RFLP methods genomic or cDNA libraries are needed. Thus the availability of suitable probes is a limiting factor.

Our orientation towards RAPD studies concerning the observation of diversity within the framework of the used cultivars have shown the advantages of using primers that have been already tested.

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Colchicine Technique of Chromosome Doubling for Soybean (*Glycine Max*) and Its Wild Relatives. First Observations on Agronomic Characteristics on *'G. Max'* Tetraploid Plants.

Introduction

Colchicine is widely largely used in chromosome doubling experiments. In *G. max,* the first experiment was reported in 1940 by Tang and Loo. The technique was then improved to increase plant survival and the frequency of recovered tetraploid plants. Colchicine has been applied on apical buds (Tang & Lin , 1963), or axillary buds. Colchicine concentrations varied from 0.3 to 0.5% in colchicine-lanoline mixtures and dropped up to 0.1% if a cotton wad saturated with an aqueous solution was used (Cheng and Hadley, 1983). To control vegetative growth of the different axes of species from *Glycine* Subgenus, Newell and Hymowitz (1979) recommended using the grafting technique.

The aim of this paper is to describe an effective procedure to obtain tetraploid plants of cultivated soybean as well as wild soybean genotypes from germinated seeds.

Material and Methods

Plant growth conditions & colchicine treatments

Seeds of *G. max* (Cultivar Goldor, Maturity Group I) and *G. canescens* (accession n° G. 1301, received from CSIRO, Canberra, Australia) were germinated on filter paper placed in petri dishes at 20 °C. After germination (hypocotyl > 10 mm), seeds were put into one of the 3 solutions containing different colchicine concentrations (2, 3, or 4 g L^{-1}) for 4 to 6 hours (C0 generation). Seeds were then washed in sterile water for 12 hours and planted out in jiffy-strips (3 cm diameter) filled with a horticultural mix. At the V3 stage, DNA content was measured and doubled plants were transferred into 4 L pots under greenhouse conditions ; they were watered once or twice a day with tap water. During winter and spring, the photoperiod was extended to 15 h with sodium vapor lamps (Philips SON T 400 W). Seeds were harvested from individual doubled plants from 3 and 4 g L^{-1} colchicine treatments. They were sown in 4 L pots placed outside (C1 generation). After cytometer analysis, tetraploid plants were placed inside insectproof cages during flowering. At the R8 stage, plants were harvested individually to produce the C2 generation.

Agronomic evaluation

For the C2 generation, seeds from Goldor derivative lines were sown in field conditions on 14 May 1997 at the Genetic and Plant Breeding Station, INRA, Montpellier, France. Each plot was two rows wide (0.45 m between rows) and 2 meters long. Plots were sprinkler irrigated when required. One plot of diploid parental line, Goldor, was included between 2 plots of experimental lines. Plant ploidy was computed from five independent measurements based on five plants sampled inside the plot. At the R8 stage, 10 plants from each plot, including the parental genotype, were sampled at random in the middle of the plots. Vegetative plant growth, node number on the main stem, pod number, number of seeds per pod and seed weight were observed and compared with parental line characteristics. Seed N content was evaluated at in the laboratory using a calibration curve generated by Near Infra Red reflectance spectroscopy (NIRSystem 6500).

DNA content measurement

DNA content was evaluated using an FACScan flow cytometer at INSERM U291 Montpellier. Plants were sampled at the V3 stage before flowering, and measurements, were assessed using 1% paraformaldehyde PBS buffer using propidium iodide fluorescence and filtered on 20 µm nylon mesh. Every 10 samples, diploid parental genotype was used as an external standard. For C0 and C1 generations, plant ploidy was estimated from two independent analyses and only one measurement for the C2 generation. A plant was considered to be a doubled plant when its DNA content was twice (± 10 %) as much as that of parental genotype.

Results and Discussion

Colchicine treatments and frequency of doubled plants

On average, the frequency of doubled plants, in the C0 generation, varied from 30% for G.1301 to 71% for Goldor. Increasing the colchicine concentration improved the proportion of doubled plants (53, 59 and 64% of plants were respectively doubled with 2, 3 and 4 g L^{-1} colchicine treatments). The proportion of mixoploid plants generally ranged from 20 to 30% ; it was maximum for the 3 g L^{-1} treatment (31%) and minimum for the 4 g L^{-1} treatment (18%). The morphology of plants was considerably modified : leaves were more or less puckered, rugose and crinkled with a dark green color, the stem had short internodes and very few seeds per plant were produced (6 seeds on average per plant).

Among 88 plants obtained in the C1 generation (Goldor derivative plants), 27 (about 30%) were actually doubled. Plants were harvested individually and, in terms of amount of seeds available, progenies of 10 genotypes were observed under field conditions (C2 generation). DNA content of 5 plants per genotype was carried out. As expected, the DNA content of experimental lines was, on average, twice as much as that of the parental line ; nevertheless there were marked of variation in DNA content between doubled plants from the same line : the coefficient of variation (C.V.) ranged from 6% to 28%. These values were

higher than these of that Goldor parental line (C.V= 3 %). This result is difficult to explain; it could be due to the difference of ploidy level within the same line, but we cannot exclude other hypotheses such as differences in DNA accessibility between plants. Nevertheless, some doubled genotypes expressed similar values for all the tested plants.

As the ploidy level could differ between the different axes present on a C0 plant for G.1301, seeds were harvested only on the doubled axis issued from 3 g L^{-1} colchicine treatment. 14 plants were obtained in the C1 generation and 11 could be considered as doubled plants; this DNA content was remarkably maintained in C2 plants.

Agronomic evaluation

At maturity, the vegetative biomass plant of doubled and diploid plants was similar (Table 1), whereas the number of nodes on the main stem was higher for doubled plants than for parental genotype (+1.5). The seed efficiency of the vegetative biomass of doubled plants (*i. e.* number of seeds divided by vegetative biomass) was significantly lower than that of parental line; consequently, seed production of doubled plants decreased as compared to diploid genotype by about 60%. The lower seed set of doubled plants was closely linked with a reduction in the number of pods harvested per plant (- 30%) due to a reduction in the number of pods produced per fertile reproductive node on the main stem (*i.e.* reproductive nodes with at least 1 pod at maturity) and with an increase in the number of sterile reproductive nodes. Moreover, the seed number per pod in Goldor doubled lines was dramatically reduced (1.18 *versus* 2.28) indicating that the colchicine treatment induced abnormal abortion of ovules or immature embryos.

According to these characteristics, as maturity approaches, the stems and the leaves of tetraploid plants remained greener longer than those of the parental genotype. Moreover, the seed weight of tetraploid plants was higher than that of diploid plants (221 *versus* 204 mg) and seed protein content of doubled plants increased : + 4.5 on average compared to parental values and remained remarkably stable between plants (standard error <1).

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Table 1: Agronomic characteristics of the Goldor parental line and derivative tetraploid lines.

	Vegetative Biomass (g/plant)	Main stem node number	Seed production (g/plant)	Pod number per plant	Seed/pod	Seed weight (mg)	Seed Protein content
Goldor n= 9	13.95 a	15,3 b	19.45 a	42.54 a	2.28 a	204 b	37.37 b
Tetraploid lines n=10	13.19 a	16.8 a	8.20 b	30.13 b	1.18 b	221 a	41.81 a

Within the same column, means with the same letter are not significantly different based on the SNK test at p=0.05; n : number of lines observed.

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Correlation and Path Coefficient Analysis of Yield and its Components in Soybean *(Glycine max* L. Merrill.)*

Abstract

Study of genotypic and phenotypic correlations in 80 genotypes of soybean *(Glycine max* (L.) Merrill.) for 13 characters indicated that seed yield had highly significant positive correlation with dry matter weight per plant. Seed yield also had significant positive correlation with number of pods per plant. Significant positive correlation was also observed between plant height and number of nodes per plant, and between dry matter weight per plant and number of pods per plant. Path analysis indicated that harvest index and dry matter weight per plant, both directly and indirectly, contributed in influencing seed yield. The results suggested that dry matter weight per plant, number of pods per plant and harvest index should be taken as the most reliable and effective selection criteria for yield improvement.

Key words: Correlation, path coefficient analysis, *Glycine max,* yield components.

Introduction

Most of the characters of breeders' interest are of complex nature and result from interaction of a number of components. Understanding the relationship between yield and yield components is of paramount importance for making best use of these relationships in selection. The correlation coefficients may be confounded with direct effects due to common association inherent in trait interrelationships. Therefore, information derived from the correlation coefficients may be augmented by partitioning correlations into direct and indirect effects by path coefficient analysis. The aim of the present study was to workout interrelationship among 13 traits in *Glycine max* and their direct and indirect effects on seed yield in diverse germplasm lines of soybean.

Materials and Methods

The experimental material comprised of 80 diverse genotypes (33 exotic, 32 indigenous germplasm lines, 11 check varieties and 4 wild type germplasm lines of unknown origin). Experimental material was sown in a randomized block design with three replications during Kharif 1995. Each plot had 3 rows of 3 m length, spaced 60 cm apart. Within row, spacing was 5 cm. Data for 13 characters were recorded on 5 randomly selected plants and averaged for statistical analysis. Correlation between 13 characters was estimated according to the method given by Searle (1961). Direct and indirect effects were estimated as described by Dewey and Lu (1959).

*A part of M.Sc. (Ag.) thesis of the first author

Results and Discussion

For selection purpose, phenotypic correlation is of little practical value unless genetic and environmental correlations between pairs of characters are in the same direction when estimated separately. Thus, the association between two traits that can be directly observed is the phenotypic correlation. The genotypic correlation in its true sense may be interpreted as the correlation of breeding value. Genetic correlation may be accounted for by linkage or pleiotropy.

The environmental correlation includes both environmental and non-additive genetic deviations. The data presented in Table 1 reveals that genotypic correlations were slightly greater in magnitude than phenotypic ones, as observed by Weber and Moorthy, 1952. This could occur when genes governing two traits are similar and the environmental conditions pertaining to the expression of these traits have small and similar effects. However, environmental correlation coefficients were low and non-significant for all the characters. Thus, phenotypic correlation coefficients would be good indices of genotypic correlation coefficients. Hence, only phenotypic correlations are referred. Moreover, characters which are genotypically but not phenotypically correlated may not be of practical value in selection since selection is generally based on phenotype.

Seed yield exhibited highly significant positive correlation (0.8658) with dry matter weight per plant. Number of pods per plant had significant positive correlation (0.5772) with seed yield, thereby indicating that dry matter weight per plant and number of pods per plant are reliable traits for improving the seed yield in soybean. These results were m line with the findings of Sichkar *et al.,* 1988, Diazcarrasco et *al.,* 1986; Yao et *al.,* 1987; Das *et al,* 1989; Plesnik, 1991; Kazmi *et al.,* 1991 and Mishra *et al.* 1994.

Number of pods per plant was significant positive correlated with dry matter weight per plant (0.6020). Number of seeds per pod was directly correlated (0.1924) with dry matter weight per plant, thus it had an indirect association with yield. Plant height showed significant positive correlation (0.6254) with number of nodes per plant. Days to flower was negatively correlated (-O.0461) with seed yield.

Seed yield exhibited positive nonsignificant correlation with plant height, number of seeds per pod, number of primary branches per plant, harvest index, number of nodes per plant, basal node height and number of nodules per plant and hundred seed weight.

The significance of dry matter weight per plant and number of pods per plant in determining seed yield became obvious through path-coefficient analysis also as these components had substantial direct effect upon seed yield (Table 2 and Table 3). Harvest index, also had substantial direct effect on seed yield. Hundred seed weight, number of nodules per plant, basal node height, number of primary branches and number of nodes per

plant do not have much direct effect on seed yield. Still, these characters determined seed yield, primarily via harvest index and dry matter weight per plant.

The findings of Malhotra *et al.* 1972; Sharma, 1979 and Das *et al.*, 1989 were in line with the positive direct effect of number of pods on seed yield. These results suggested that selection for traits directly related to yield may be advantageous in terms of bringing direct effect on yield. For the characters that are indirectly associated with yield, the indirect effect on yield may be observed via improvement in directly associated characters. Residual error showed that there could have been some more characters responsible for influencing the seed yield.

Thus, during the process of selection in the germplasm of soybean, it is suggested that dry matter weight per plant and number of pods per plant should have prime consideration for yield improvement. Since it is easier to record unthreshed weight than non-seed dry matter weight and in view of its higher correlation with seed yield, more emphasis should be placed on unthreshed weight in the selection process.

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Table 1. Phenotypic (in bold) and Genotypic coefficient of correlation among different character in Soybean.

Characters	Days to flower	Days to maturity	Plant height (cm)	Number of pods per plant	Number of seeds per pod	Number of primary branches per plant	Harvest index	Number of nodes per plant	Basal node height (cm)	Number of nodules per plant	100-seed weight (g)	Dry matter weight per plant (g)	Seed yield per plant (g)
1. Days to flower	**1.00**	**0.5307**	**0.2204**	**-0.1010**	**-0.2011**	**-0.0719**	**-0.0937**	**O.0967**	**0.1146**	**-0.3576**	**-0.2910**	**-0.0209**	**-0.0461**
	1.00	0.5339	0.2274	-0.1013	-0.2156	-0.0725	-0.0951	O.1004	0.1178	-0.3602	-0.3174	-0.0221	-0.0471
2. Days to maturity			**0.3723**	**0.1180**	**0.0947**	**-0.1678**	**-0.2046**	**O.098**	**0.0316**	**-0.2568**	**-0.1994**	**0.1265**	**0.0304**
			0.3909	0.1190	0.1029	-0.1712	-0.2085	O.0996	0.0339	-0.2602	-0.2157	0.1266	0.0279
3. Plant height (cm)				**0.2518**	**0.1540**	**-0.1215**	**-0.4592**	**O.6254***	**0.0380**	**-0.1934**	**-0.2753**	**0.4014**	**0.1795**
				0.2661	0.1805	-0.1319	-0.4897	O.6715	0.0451	-0.2022	-0.3131	0.4218	0.1851
4. Number of pods per plant					**0.2129**	**0.3669**	**-0.1083**	**O.4271**	**0.0457**	**-0.0995**	**-0.2171**	**0.6020***	**0.5772***
					0.2234	0.3731	-0.1095	O.4388	0.0456	-0.1006	-0.2337	0.6077	0.5821
5. Number of seeds per pod						**0.2317**	**0.1309**	**O.0799**	**0.0845**	**0.1645**	**0.0047**	**0.1924**	**0.2733**
						0.2470	0.1424	O.0852	0.0936	0.1758	0.0145	0.2081	0.2943
6. Number of primary branches per plant							**0.1514**	**O.1141**	**-0.0612**	**0.0555**	**-0.0849**	**0.1604**	**0.2693**
							0.1545	O.1144	-0.0595	0.0568	-0.0961	0.1617	0.2727
7. Harvest index								**-0.3934**	**0.2274**	**0.2447**	**0.4519**	**-0.3278**	**0.1733**
								-0.4026	0.2309	0.2466	0.4884	-0.3300	0.1799
8. Number of nodes per plant									**-0.1048**	**-0.0804**	**-0.2643**	**0.4842**	**0.3005**
									-0.1085	-0.0829	-0.2932	0.4930	0.3060
9. Basal node height (cm)										**-0.0468**	**0.0165**	**0.0447**	**0.1646**
										-0.0493	0.0605	0.0464	0.1667
10. Number of nodules per plant											**0.2713**	**0.0821**	**0.0253**
											0.2897	0.0823	0.0254
11. 100-Seed weight (g)												**-0.0287**	**0.1835**
												-0.0313	0.1971
12. Dry matter weight (g)													**0.8658****
													0.8678
13. Seed yield per plant (g)													**1.00**
													1.00

*Significant at 5 per cent level of probability
**Significant at 1 per cent level of probability

Table 2. Genotypic path coefficient analysis of seed yield with other characters in Soybean.

Characters	Days to flower	Days to maturity	Plant height (cm)	Number of pods per plant	Number of seeds per pod	Number of primary branches per plant	Harvest index	Number of nodes per plant	Basal node height (cm)	Number of nodules per plant	100-seed weight (g)	Dry matter weight per plant (g)
1. Days to flower	**0.3912**	-0.0201	0.0455	-0.1023	0.0149	-0.0071	0.0213	0.0204	0.0213	-0.2578	-0.1879	0.0134
2. Days to maturity	0.2089	**-0.0376**	0.0782	0.1202	-0.0071	-0.0168	0.0466	0.0202	0.0061	-0.1862	-0.1277	-0.00770
3. Plant height (cm)	0.0889	-0.0147	**0.2001**	0.2688	-0.0125	-0.0129	0.01095	0.1363	0.0081	-0.1447	-0.1853	-0.2567
4. Number of pods per plant	-0.0396	-0.0045	0.0533	**-1.0102**	-0.0155	0.0365	0.0245	0.0891	0.0082	-0.0719	-0.1383	-0.3698
5. Number of seeds per pod	-0.0843	-0.0039	0.0361	0.2257	**-0.0693**	0.0242	-0.0319	0.1727	0.0169	0.1258	0.0086	-0.1266
6. Number of primary branches per plant	-0.0284	0.0064	-0.0264	0.3769	-0.0171	**0.0979**	-0.0346	0.0232	-0.0107	0.0406	-0.569	-0.0984
7. Harvest index	-0.0372	0.0078	-0.0979	-0.1106	-0.0099	0.0151	**-0.2237**	-0.0817	0.0417	0.1765	0.2890	0.2008
8. Number of nodes per plant	0.0393	-0.0037	0.1344	0.4433	-0.0590	0.0112	0.0901	**0.2030**	-0.0196	-0.059	-0.1735	-0.2999
9. Basal node height (cm)	0.0461	-0.0013	0.0090	0.0461	-0.0065	-0.0058	-0.0516	-0.0220	**0.1805**	-0.0353	-0.0358	-0.0282
10. Number of nodules per plant	-0.1409	0.0098	-0.0405	-0.1016	-0.0122	0.0056	-0.0552	-0.168	-0.0089	**0.7156**	0.1715	-0.5009
11. 100-Seed weight (g)	-0.1242	0.0081	-0.0627	-0.2361	-0.0010	-0.0094	-0.1092	-0.0595	0.0109	0.2073	**0.5919**	-0.0190
12. Dry matter weight (g)	-0.0086	-0.0048	0.0844	0.6139	-0.0144	0.0158	0.0738	0.1001	0.0084	0.5892	0.0185	**-0.6085**

Residual factor = +0.7271
Diagonal values (in bold) represent direct effect.

Table 3. Phenotypic path coefficient analysis of seed yield with other characters in Soybean.

Characters	Days to flower	Days to maturity	Plant height (cm)	Number of pods per plant	Number of seeds per pod	Number of primary branches per plant	Harvest index	Number of nodes per plant	Basal node height (cm)	Number of nodules per plant	100-seed weight (g)	Dry matter weight per plant (g)
1. Days to flower	**-0.0660**	-0.584	0.0199	0.0781	0.0009	0.0128	-0.1465	-0.0414	-0.1634	0.2325	0.1436	-0.0571
2. Days to maturity	-0.0355	**-0.1101**	0.0336	-0.0912	-0.0004	0.0298	-0.3199	-0.0420	-0.0451	0.1669	0.0984	0.3458
3. Plant height (cm)	-0.0148	-0.0409	**0.0903**	-0.1947	-0.0007	0.02159	-0.7180	-0.2681	-0.542	0.1257	0.1358	1.0974
4. Number of pods per plant	0.0068	-0.0129	0.0227	**-0.7732**	-0.0009	-0.0652	-0.1693	-0.1831	-0.0652	0.0647	0.1071	1.6459
5. Number of seeds per pod	0.0135	-0.0104	0.0139	0.1646	**-0.0045**	-0.0412	0.20479	-0.0342	-0.1205	-0.1069	-0.0023	0.5260
6. Number of primary branches per plant	0.0048	0.0185	-0.0109	-0.2837	-0.0010	**-0.1777**	0.2367	-0.0489	0.0873	-0.0361	0.0419	0.4385
7. Harvest index	0.0063	0.0225	-0.0415	0.0837	-0.0006	-0.0269	**1.5636**	0.1686	-0.3243	-0.1591	-0.2229	-0.8962
8. Number of nodes per plant	-0.0065	-0.0108	0.0565	-0.3302	-0.0004	-0.0203	-0.6151	**-0.4286**	0.1495	0.0523	0.1304	1.3238
9. Basal node height (cm)	-0.0077	-0.0035	0.0034	-0.0353	-0.0004	0.0198	0.3556	0.04490	**-1.4261**	0.0304	-0.0303	1.2226
10. Number of nodules per plant	0.0239	0.0263	-0.0175	0.0769	-0.0007	-0.0099	0.3826	0.0345	0.0667	**-0.6502**	-0.1338	0.2246
11. 100-Seed weight (g)	0.0195	0.0219	-0.0249	0.1679	-0.00002	0.01514	0.7066	0.11335	-0.0877	-0.1764	**-0.4933**	-0.0785
12. Dry matter weight (g)	0.0014	-0.0139	0.0363	-0.4655	-0.0009	-0.0285	-0.5126	-0.2075	-0.6377	-0.0534	0.0142	**2.7339**

Residual factor = +0.6881

Diagonal values (in bold) represent direct effect

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Evaluation of Soybean Germplasm for Seed Oil Content

Introduction

Soybean [*Glycine max* (L.) Merrill] tops in the world production of both oilseeds and edible oils. It is grown in United States, China, Brazil and Argentina mainly for oil while the deoiled cake is used as cattle feed and fertilizer. Soybean contains about 20% cholesterol free oil, 40% good quality protein, 23% carbohydrates and reasonable amounts of minerals and vitamins. In the germplasm lines oil percentage ranges from 7% in *Glycine soja* to nearly 28% in *Glycine max* (Palmer et al. 1996).

Soybean is also an important oilseed and pulse crop in India. At present, soybean occupies third place among the nine-oilseed crops of India. During the last two decades, about 40 soybean varieties have been bred in India. The high yield potential of these varieties has resulted in increase in the area under their cultivation. Present area under soybean in India is estimated to be around 5.0 million hectares with a production of about 4.60 million tons and a productivity level of 920 kg/ha. The state of Madhya Pradesh has about 70% of total soybean area in the country followed by Maharashtra (19.72%) and Rajasthan (4%). Our centre has been actively engaged in germplasm maintenance, characterization, evaluation, documentation, and conservation activities since its inception in 1968. The main object of this centre is to identify and select early to midlate maturing varieties suitable for growing in rainy season. So far, five soybean varieties, viz. Monefta, MACS-1 3, MACS -57, MACS-58 and MACS-1 24 have been released from this Institute for their commercial cultivation in Central and Southern India.

Germplasm is the most valuable and essential basic raw material needed to meet the current and future needs of any crop improvement programme. Crop germplasm with wide genetic base is very important to develop new high yielding varieties along with high oil content. Hence, this institute has initiated work on the screening of available soybean germplasm lines for seed oil content, which will be useful in future breeding programme.

Materials and Methods

A field experiment was conducted at Agharkar Research Institute's nursery farm at Pune during rainy seasons of 1992 and 1993 in augmented block design using MACS-58 as check variety (sown after every 20 lines) which contains 21.5% seed oil. Soil is medium black with pH 7.5 and the average annual rainfall is about 600 mm. A basal dose of NPK @ 20:80:40 kg/ha was given before sowing in the form of ammonium sulfate, single super phosphate and muriate of potash. Five hundred and sixty-seven soybean germplasm lines of diverse origin and different maturity groups, viz. early (maturing within 90 days), midlate (90-100 days) and late (more than 100 days) collected from all over the world were sown in the last week of June and harvested during September to October. Seed of each accession was sown in a single row of one meter length with a spacing of 45 cm x 5 cm between rows and plants, respectively. Seed oil content was estimated on Oxford NMR 4000 Analyser. The data were analysed using standard statistical procedures.

Results and Discussion

Variability in Maturity groups

Variability for seed oil content in 567 soybean germplasm lines in different maturity groups is given in Table 1.

The data presented in Table 1 indicated wide range of variability for seed oil content. The oil content ranged from 13.82% in 'PLSO-101' to 24.42% in 'Lee' variety. The early maturing varieties showed wide range of oil content (13.82 to 24.42%) than the midlate (15.08 to 22.79%) and late (14.82 to 21.18%) maturing varieties. Jaipurkar and Thakare (1981) have also recorded 17.90% to 23.70% oil content, minimum being in Monetta and maximum being in EC.9308. Similar results were obtained by Tripathi *et al.* (1975) and Bharambe and Bodhe (1976). Wilson (1987) has reported a range of 13.3% to 27.6% oil content minimum being in PI 408042 and maximum being in PI 371611. Recently Billore and Joshi (1997) have also reported that early maturity group contained comparatively higher oil content than those under normal maturity group. In their study the highest oil content was exhibited by NRC-7 (19.78%) and lowest by Punjab-1 (12.54%).

Variability in oil content with different seed coat colour

Seed coat colour is an economically important character as there is preference for yellow seeded varieties both by consumer as well as industry. Normally soybean seeds exhibit wide range of variations in seed coat colours and pattern. They may be yellow, green, brown, black or buff mainly located in the outer layer of seed coat. Oil content in different seed coat coloured varieties is given in Table 2.

It can be seen from the above table that the range of oil content is more in yellow seed coat coloured seeds (15.08 to 24.4%) which was followed by brown seeds (13.82 to 21.7%), black seeds (14.36 to 20.3%) and green seeds (15.64 to 21.2%). Likewise, highest average oil content was observed in the accessions with yellow seed coat colour (19.12%), followed by green (18.28%) black (18.21%) and brown (17.49%) seed coat colours. The highest average oil content recorded in the accessions with yellow seed coat might be attributed to extensive breeding efforts made for genetic improvement in this character. Mohamed *et al.* (1990) also reported a range of 17.24 to 23.36% oil content with an average of 18.44%.

The genotypes having more than 21.5% oil content are given in Table 3.

Present studies indicated that 21 accessions out of 567 recorded higher oil content than the check variety MACS-58 (21.5% oil). Out of these, above 11 accessions may be useful in future breeding programmes for increasing oil content in soybean.

Conclusions

Present studies indicated that early maturing varieties have comparatively higher oil content than midlate and late maturing varieties. Likewise, varieties with yellow seed coat have relatively more oil content followed by green, black and brown seed coat colours. Soybean varieties, viz., EC.16695 (Lee), EC.62817, EC.77207, EC.95807, EC.100029, EC.96048, EC.100026, EC.95291, EC.175325, IC.7217, and MACS-58 have high oil content and these varieties may be used in breeding programme for future crop improvement.

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Table 1. Variation in oil content in different maturity groups.

Sr. No	Maturity groups	No. of Lines tested	Range (%)	Mean ± S. E. (%)	C. V. %
1.	Early (<90 days)	205	13.82 – 24.42	18.57 ± 0.4	10
2.	Midlate (90 – 100 days)	234	15.08 – 22.79	19.17 ± 0.10	8
3.	Late (>100 days)	128	14.82 – 21.18	18.59 ± 0.10	6
	Total	567	13.82 –24.42	18.82 ± 0.07	9

Table 2. Variability for oil content based on seed coat colour.

Sr. No	Seed coat colour	No. of Lines tested	Range (%)	Mean ± S. E. (%)	C. V. %
1.	Yellow	376	15.08 – 24.42	19.12 ± 0.08	8
2.	Green	49	15.64 – 21.25	18.28 ± 0.28	10
3.	Brown	115	13.82 – 21.74	17.49 ± 0.26	7
4.	Black	27	14.36 – 20.38	18.21 ± 0.12	8
	Total	507	13.82 – 24.42	18.82 ± 0.07	9

Table 3. Accessions with high oil content.

Sr. No.	MACS (ARI) P.I. No.	IARI New Delhi No.	Source / Origin	Average oil Content (%)
1.	4247	EC. 16695 (Lee)	USA	24.42
2.	2549	EC. 62817	Japan	23.00
3.	1953	EC. 77207	USA	22.79
4.	2614	EC. 95807	USA	22.66
5.	4147	EC. 100029	Ghana	22.50
6.	2667	EC. 96048	USA	22.47
7.	4145	EC. 100026	Ghana	22.47
8.	2585	EC. 95291	USA	22.42
9.	4979	EC. 175325	USA	22.37
10.	360	IC. 7217	India	22.01
11.	MACS-58	-	India	21.50

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Field Weathering in Soybean

Introduction

Although field-weathering effects under tropical conditions are well known for soybean (Bhatia et al. 1996), information regarding extent of field weathering under natural conditions suffered by different Indian soybean varieties were not available. During rainy season of 1997, soybean plants left unharvested for about a month received intermittent rains. This alternate wetting and drying exposure had created a very favourable condition for field weathering and varietal differences with reference to the extent of natural field weathering of seed was studied.

Materials and Methods

During rainy season of 1997 at Indore, 33 varieties of soybean were grown in the field. Each variety was grown in 5 plant to progeny row plots. At the crop maturity, a part of the plot was harvested. For each variety, 3 randomly selected 1 m long rows were left unharvested for field weathering and pod shattering observations. The mature plants received intermittent rain for about a month and hence were repeatedly exposed to alternate wetting and drying. The environment was very unusual but ideal for natural field weathering of soybean seeds. These field-weathered plants were harvested when dry and seeds were threshed. Seeds received from timely harvest and seeds received after natural field weathering were put for germination tests.

Results and Discussion

Based on the extent of reduction in germination due to field weathering, these varieties could be grouped into 3 distinct categories. In the first category, there are 13 varieties, which did not show any significant reduction in seed germination due to field weathering. These varieties are JS 335, JS 80-21, JS 79-81, JS 76-205, MACS 13, Hardee, Lee, PK 471, Pusa 22, Pusa 37, Birsa Soybean 1, Gujarat Soybean 1, and VLS 2. The second category consists of 6 varieties showing up to 10% reduction in germination due to field weathering. These varieties are Ankur, Gujarat Soybean 2, JS 75-46, KHSb-2, PK 308 and PK 416. In the third category, varieties showing more than 10% reduction in germination are grouped. These 14 varieties are Bragg, MUAS 1, NRC 2, NRC 12, PK 262, PK 471, PK 564, PK 1024, PK 1027, PK 1042, Pusa 16, Pusa 20, Pusa 24, and Pusa 40. Thus most of the important Indian soybean varieties currently in the seed chain are prone to field weathering. The exceptions are JS 335, JS 80-21, JS 79-81, and MACS 13 and hence they can be used as donor parent in breeding programme.

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Genetic Diversity Among Indian Soybean Varieties

Introduction

For development of improved soybean varieties with higher yield potential through hybridization, sufficient genetic divergence for agronomic traits among parents is necessary. Accordingly, identification of genetically diverse parents among elite varieties is important for obtaining heterotic F1 and broad spectrum variability in segregating generations (Arunachalam, 1981). In soybean, crosses involving moderate to highly diverse parents showed heterosis in F1 and also greater variability in F2 (Chauhan and Singh, 1982). Soybean breeders have used Mahalanobis D^2 to measure the extent of genetic diversity within germplasm or in segregating generations of crosses (Shwe et al., 1972, Bains and Sood, 1984, Singh and Ram, 1985, Sharma et al., 1986, Misra et al., 1987, Tawar et al. 1987, Chikhale et al., 1992). Therefore, the present study was undertaken to assess the extent of genetic diversity for agronomic traits existing among the elite Indian soybean varieties for selection of genetically diverse parents for hybridization.

Materials and Methods

Forty-one varieties of soybean identified for cultivation in different parts of India were evaluated during rainy seasons of 1991 and 1992 at Indore, Madhya Pradesh. The trials were conducted in RBD with three replications. Each variety was sown in 6 rows of 4 m length, at 45 cm apart. Observations were recorded on days to flowering, maturity, yields of seed, straw and leaf litter on plot basis and plant height, pods/plant on five random plants. Analysis of variance for different characters was done as per standard procedure (Panse and Sukhatme, 1978). Mahalanobis D^2 and Canonical analysis were carried out using the pooled data and the varieties were grouped on the basis of minimum generalized distance using Tocher's method as described by Rao (1952).

Results and Discussion

Highly significant differences were observed among the varieties for all the characters. The D^2 values among the varieties varied from 4.73 to as high as 3660.2. On the basis of relative magnitude of D^2 values, the varieties were grouped into six clusters (Table 1). Ten varieties were accommodated in cluster I, 20 in cluster II, 5 in cluster III, 4 in cluster IV and one each in clusters V and VI. Maximum genetic divergence was observed between clusters V and VI (60.5) followed by clusters IV and VI (50.6) and clusters III and V (47.8) (Table 2). Least divergence were observed between clusters I and II (11.4) and between clusters IV and V (11.9) followed by clusters I and III (13.9), clusters III and VI (15.7) and clusters II and IV (17.5). The intracluster divergence were low and varied between 7.1 and 8.1. The canonical analysis confirmed the clustering pattern obtained by D^2 analysis. The cluster means for different characters are given in Table 3.

The present study shows that about 75 percent of the varieties fall under two genetically less divergent clusters (I and II) characterized by a moderate and probably balanced expression of the economically important characters. No parallelism was observed between grouping of varieties and their zone of adaptation/cultivation. Earlier studies also indicated lack of parallelism between geographic adaptation and genetic diversity (Shwe et. al, 1972, Sharma et. al, 1986).

These soybean varieties represent the most adapted genotypes of soybean in India and hence can serve as source of elite parents for varietal improvement through hybridization. These varieties to be used as parents should possess superior agronomic characteristics and should also be diverse enough to yield superior segregants (Arunachalam, 1981). Maximum genetic divergence was observed between varieties JS 71-05 and T 49. In soybean, even moderately diverse parents were reported to produce highly variable progeny in segregating generation (Chauhan and Singh, 1982). Hence hybridization of varieties JS 2 and Monetta (cluster IV) with JS 71-05 (cluster V) may yield superior segregants for earliness. Hybridization of varieties PK 262 (cluster 1), PK 308, PK 327, PK 471, and PK 564 (cluster 11) with PK 416 (cluster IV) may result in desirable recombinants for high yield. Simultaneously selection should be practiced for resistance to pod shattering and good seed viability under ambient storage.

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Table 1. Composition of genetically divergent clusters in soybean

Cluster No.	No. of Varieties	Varieties included in the cluster
I	10	Pusa 24, Alankar, Pusa 20, PK 262, MACS 13, MACS 57, JS 80-21, Durga, Gujarat Soy 1, Gujarat Soy 2
II	20	Pusa 40, VLS 2, VLS 1, Shilajeet, Shivalik, PK 471, Pusa 16, Ankur, PK 564, Birsa Soy 1, Gaurav, Pusa 37, PK 308, JS 75-46, PK 472, Kalitur, MACS 58, Hardee, Punjab 1, PK 327
III	5	Pusa 22, KHSb 2, Lee, Imp. Pelican, Co 1
IV	4	JS 2, PK 416, Bragg, Monetta
V	1	JS 71-05
VI	1	Type 49

Table 2. Average intracluster (in bold) and intercluster D^2 and D values

Clusters	I	II	III	IV	V	VI
I	50.30 (7.1)	129.1 (11.4)	193.4 (13.9)	670.2 (25.9)	1274.6 (35.7)	689.2 (26.2)
II		56.4 (7.5)	503.5 (22.4)	305.5 (17.5)	723.6 (26.9)	1234.4 (35.1)
III			64.2 (8.0)	1424.3 (37.7)	2283.9 (47.8)	247.3 (15.7)
IV				65.1 (8.1)	141.9 (11.9)	2563.0 (50.6)
V					-	3660.2 (60.5)
VI						-

Figures in parenthesis are D values.

Table 3. Cluster means for different characters

Cluster No.	Seed yield (q/ha)	Straw yield (q/ha)	Leaf litter yield (q/ha)	Pods per plant	Plant height (cm)	Days to flowering	Days to maturity	Apparent biomass (q/ha)	Total biomass (q/ha)	Apparent harvest index (%)	Real harvest index (%)
I	17.59	36.07	11.96	66.46	87.30	46.65	105.97	53.81	65.76	33.07	27.00
II	19.53	33.68	11.95	62.57	75.19	45.42	102.18	53.73	65.85	36.52	29.89
III	13.07	36.79	13.44	69.45	108.89	49.33	108.00	51.84	63.30	26.08	21.09
IV	20.28	36.33	10.94	53.13	68.34	37.92	98.29	56.60	69.90	36.55	30.58
V	18.59	27.79	7.71	51.23	43.00	34.33	99.00	46.37	54.80	40.02	34.18
VI	10.04	36.94	22.90	84.13	100.33	55.17	122.83	46.15	69.05	21.97	14.43

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Identification of Bold Seeded Soybean Genotypes with Good Seed Longevity in Segregating Generations of Crosses

Introduction

Availability of seed of improved varieties of soybean with high germination percentage to ensure adequate plant stand is a major constraint in harnessing their yield potential in India. Poor seed viability could be due both to genetic and adverse environmental factors. Bold-seeded Indian varieties are generally prone to rapid seed deterioration, whereas many small seeded varieties have good seed longevity (Tiwari and Joshi 1989). Decline in seed viability after 7 months of storage of bold seeded soybean varieties in particular is of major concern. Bold seeded lines retaining at least 70% seed viability after 8-9 months of ambient storage if developed can improve the yield potential of soybean varieties in India. Hence, the relationship between seed size and seed viability in segregating generations of crosses was studied to explore the possibility of identify bold--seeded lines with good seed longevity.

Material and Methods

Seeds were harvested from 67 progenies of F6, F7 and F8 generations derived from 16 crosses involving 16 varieties with varying seed size and seed longevity, and from 4 check varieties JS 335, PK 472, NRC 12 and JS 71-05 grown during rainy season of 1996. Seeds were cleaned and stored under ambient conditions for 10 months. 100 seed weight and- germination percentage was recorded from these 10 months old seeds. Lines were selected having bold seed as well as good seed longevity.

Results and Discussions

Data on mean and range of 100 seed weight and germination (%) in different crosses are presented in Table 1. The correlation between 100 seed weight and germination (%) was not significant (r = -0.06). Two selections from the cross JS 335 x Bragg (F6) and 3 selections from the cross JS 80-21 x PK 472 (F6) had bold seeds and retained more than 70% germination after 10 months of ambient storage under tropical condition (Table 2). Crossing among these selections followed by screening of segregating progenies for identification of bold seeded lines with further improved seed longevity is required.

Acknowledgement

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Table 1. Seed weight and seed germination in segregating generations of crosses of soybean after 10 months ambient storage.

Cross	No. of Progenies	100 Seed Weight (g)		Germination %	
		Mean	Range	Mean	Range
Pb 1 x Monetta F6	6	11.8	11 to 13	75.1	64.3 to 88
JS 335 x Pb 1 F6	4	13.4	12.3 to 14.6	76.0	71 to 81
JS 80-21 x Pb 1 F6	2	13.7	11 to 16.5	49.3	29 to 69.6
Gaurav x Pb 1 F6	4	11.9	9.3 to 16.5	70.9	66.3 to 75.6
PK 308 Segregants	1	14.0	14.0	71	71
Pb 1 x G 303 F6	1	12.6	12.6	91.6	91.6
Pb 1 x G 219 F6	2	12.1	9.6 to 14.6	82.8	80.6 to 85
JS 335 x Pusa 20 F6	3	13.2	12.6 to 14.0	61.9	48 to 76.6
JS 335 x Bragg F6	8	15.3	14.6 to 16.0	73.1	53.6 to 92.5
JS 335 x MACS 58 F6	5	12.7	11.33 to 14.6	53.4	43.0 to 73.6
PK 472 x JS 80-21 F6	1	14.3	14.3	60.3	60.3
Bragg x L 129 F6	17	13.6	10.6 to 15.0	77.6	76.3 to 89.6
PK 472 x NRC 1 F8	1	13.0	13.0	34.5	34.5
PK 472 x L 129 F6	2	13.1	13.0 to 13.3	75.6	74.3 to 77.0
JS 75-46 x NRC 1 F7	6	15.6	15.0 to 16.5	41.0	31.0 to 53.0
JS 80-21 x PK 472 F6	4	16.1	15.6 to 16.5	75.7	72.5 to 83.3

Table 2. Promising bold seeded selections retaining good seed longevity after 10 months of ambient storage

Cross	Selections	100 seed weight (g)	Germination (%)
JS 335 x Bragg (F6)	1	16.0	83.6
	2	16.0	77.6
JS 80-21 x PK 472 (F6)	3	16.0	72.6
	4	16.5	74.5
	5	16.5	72.5
Check Varieties			
JS 335		11.8	53.9
PK 472		11.5	60.5
NRC 12		12.5	69.5
JS 71-05		16.7	46.7

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Metroglyph and Index Score Analysis in Advanced Breeding Lines of Soybean

Abstract

Thirty advanced breeding lines of soybean derived from ten different single crosses were utilized for genetic divergence study by using Metroglyph analysis. Two highly variable characters viz. Pods/plant and yield/plant were taken such that the X coordinate represented yield/plant, while the Y coordinate represented pods/plant. Five other characters viz., 100 seed weight, seeds/pod, harvest index, reproductive phase and dry matter were represented by rays at different positions on the glyph. All the genotypes were grouped into 7 different clusters where the constellation of the genotypes was based not only on the position of the glyph but on the pattern of the rays. A judicious categorization was obtained through scattered diagram. Cluster I and II which were represented 10 and 5 genotypes respectively had the most important genotypes.

Key words: Genetic divergence, Metroglyph analysis

Genetic variability is the basic requirement for making progress in crop breeding. Inclusion of genetically diverse parents in a breeding programme, is essential to create new genetic stock. Earlier workers showed the importance of genetic divergence for yield and yield attributes (1, 2). Chandra (4) has suggested that metroglyph analysis would be more suitable for preliminary grouping before taking up D^2 analysis. In the present investigation, genetic diversity based on Metroglyph analysis in a set of 30 soybean breeding lines which were free from yellow mosaic virus and bacterial pustules was assessed for yield and yield attributes.

Materials and Methods

Thirty soybean breeding lines (28 advanced breeding lines, i.e. PK 1138-PK 1166) and two check varieties viz. PS 564 and PK 327 were evaluated during Kharif 1994 in a randomized block design with 4 replications adopting the spacing of 60 × 5 cm. Each genotype was grown in 5 rows of 4m length. Mean values of 5 plants chosen at random from the central row of each plot were used for statistical analysis. The metroglyph and index score analysis were carried out as per Anderson (1). Out of seven traits, two highly variable characters viz., pods/plant (c.v. = 25.34%) and yield/plant (c. v. = 27.59%) were taken such that the X coordinate represented yield/plant while the Y coordinate represented pods/plant. Five other traits viz., 100-seed weight, seeds/pod, harvest index, reproductive phase, and dry matter were represented by rays at different positions on the glyph (Table 1) were plotted for each genotype against the X and Y coordinates based on their plant yield and pods/plant. All the breeding lines were represented by open circles (glyph), while both the check varieties were represented by dark solid circles. The range in each character included in this analysis except for yield/plant and pods/plant has been classified into 3 classes, each represented by different lengths at the same position on each glyph.

Results and Discussion

Results of the metroglyph analysis are presented in Fig. 1. The scattered diagram revealed that seven groups could be distinguished in these characters. The first group is represented by 10 genotypes. This cluster, based on ray pattern, may be characterized for high yield/plant, moderate number of seeds/pod, generally long reproductive phase, low dry matter, high harvest index, and high 100-seed weight.

The second cluster, represented by 5 genotypes, has,in general, genotypes with high harvest index, high 100-seed weight, greater number of pods/plant, more dry matter and a long reproductive phase. The third cluster also included 5 genotypes. All the genotypes in this cluster were moderate for yield/plant and pods/plant. Generally low 100-seed weight, smaller pods, moderate dry matter, medium reproductive phase and medium to high harvest index can be observed with the help of rays.

The fourth cluster carries 4 genotypes which could be distinguished based on a ray pattern showing low harvest index, 100-seed weight, more pods/plant, less dry matter, and short reproductive phase.

The fifth cluster is represented by 3 genotypes, including check variety PK 327 which was characterized by high harvest index, low 100-seed weight, more seeds/pod, more dry matter, and low reproductive phase.

The two genotypes in cluster six had high pods/plant, and also very high yielding potential while the ray pattern suggested the presence of 100-seed weight, less pods/plant, high dry matter, medium reproductive phase, and low harvest index.

The seventh cluster is represented by a single genotype. This genotype is a little bit similar to the genotype of the first cluster but due to high pod number, moderate yield and low harvest index, it was grouped into a separate cluster that included medium 100-seed weight, moderate number of seeds/pod, high dry matter and medium reproductive phase.

The grouping of genotypes into different clusters, along with the main characteristic features of each cluster, is presented in Table 2. The constellation of lines was based not only on the position of the glyph but also on the pattern of the rays. Attention was paid to the fact that no glyph with big differences in ray pattern, was included in a cluster where the other glyphs followed an average common ray pattern. It was observed that with respect to pod number and yielding ability within groups, differences were not high except for the fourth cluster which showed less within group differences for yield but these differences were high for pods/plant. Even though the peculiar similarity in the ray pattern among the member genotypes could

not be ignored, hence they are grouped together. For the rest of the genotypes, the ray pattern was almost similar.

Figure 1 indicates that donors for different characters viz. high yield (II and VI), high pods/plant (V and VI), long reproductive phase (I and II), short reproductive phase (V), high harvest index (I and II), low harvest index (VII and IV), high 100-seed weight (I and II), low 100-seed weight (V), greater number of seeds/pod (V), less number of seeds/pod (III), high dry matter (VI) and low dry matter (III and IV) can be selected from the respective clusters. Cluster I and II expressed the most important genotypes.

To quantify the divergence, index score analysis was carried out and an index score diagram was proposed (Fig. 1). Though no distinct class could be obtained, the frequency distribution around different score values showed the overall nearness or divergence between genotypes. High frequencies around a particular index value (i.e. values 13, 16 & 19 in Fig. 1) suggest closeness among the genotypes crosses between genotypes near the 2 extreme index values may give better heterotic combinations. The greater range of index values suggests that there is more variability between these lines (Ramanujam and Kumar (2)).

Conclusions

Metroglyph analysis is based mainly on 2 characters plotted as X– and Y– coordinates and therefore is subjective (Chandra (4)). In this technique the precision of classification depends on the characters explained by the coordinates, which means to get higher precision it is important that maximum variability be expressed by these characters, hence, the two characters should be chosen judiciously (Chhabra et al. (3)).

Based on the present analysis, it can easily be concluded that the genotypes having most of the important characters were present in cluster I and II. The large range for certain characters suggest high variability among genotypes.

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Table 1: Index Scores (Range) for different characters studied in thirty advanced breeding lines of soybean

S1. No.	Character	Range of Mean	Score I (1) Value less than	Sign	Score II (2) Value from- to-	Sign	Score III (3) Value more than	Sign
1.	Seeds/pod	2.08-2.53	2.23	o	2.23 to 2.27		2.27	
2.	100-seed weight	6.72-11.38	10.00	o	10.00 to 10.60		10.60	
3.	Harvest index	0.32-0.477	0.43	o	0.43 to 0.44		0.44	
4.	Reproductive phase	56.00-69.00	64.00	o	64.00 to 67.00		67.00	
5.	Pods/plant	78.14-169.9	110.00	y-axis	109.0 to 120.0	-	120.00	-
6.	Yield/plant (g)	13.34-28.80	19.00	x-axis	19.0 to 24.00	-	24.00	-
7.	Dry matter	29.00-70.00	45.00	o	45.0 to 55.00		55.00	

Table 2: Mean cluster values of seven characters included in the Metroglyph Analysis

S. No.	Characters	Cluster I	II	III	IV	V	VI	VII
1.	Pods/plant	110.46	122.85	95.95	103.39	164.40	149.26	127.65
2.	Plant yield (g)	23.72	27.11	19.29	14.04	22.44	27.06	22.99
3.	Reproductive phase (days)	66.80	67.50	65.95	63.12	60.25	65.75	66.00
4.	Harvest index	0.435	0.445	0.433	0.365	0.433	0.432	0.392
5.	100-seed weight (g)	10.51	10.53	10.28	9.31	9.04	9.76	10.28
6.	Seeds/pod	2.26	2.28	2.18	2.26	2.35	2.21	2.25
7.	Dry mater	54.80	59.75	46.02	37.78	53.00	63.50	55.25

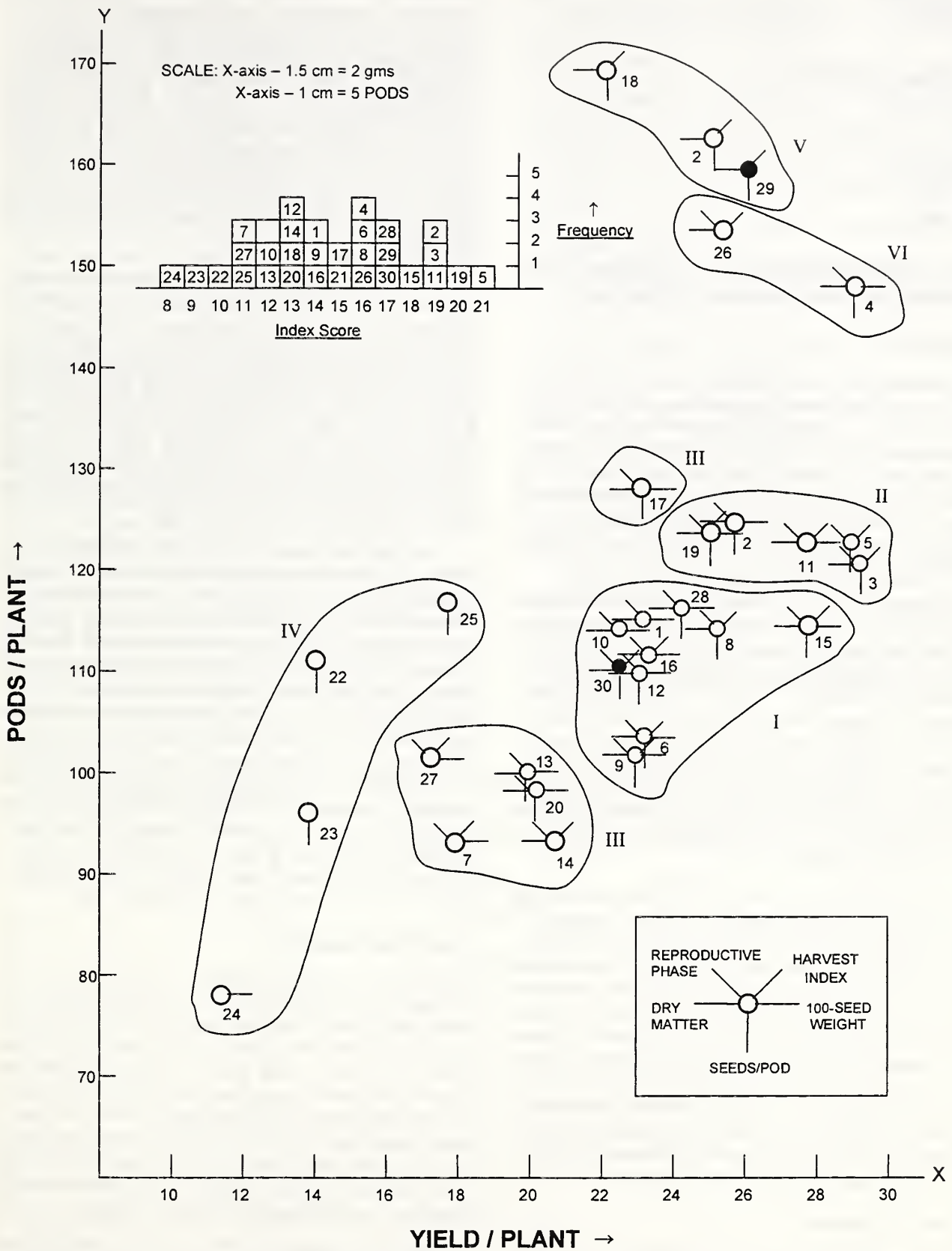

Fig. 1: Metroglyph and index score analysis in advanced breeding lines of soybeans.

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Phenylalanine Ammonialyase in Shattering And Shattering Resistant Genotypes of Soybean

Introduction

Pod shattering is a serious problem in several varieties of soybean in the tropical and subtropical regions and has proved to be a major cause of yield erosion (Tiwari and Bhatnagar, 1991). Pod shattering is referred to as the opening up of pods and dispersal of seeds at the time of maturity before as well as during harvesting of the soybean crop. The percentage of shattering varies in the varieties ranging from negligible to as high as 30%. Normally a pod shatters due to moisture loss and mechanical force exerted by the pod wall.

Biochemical studies in Brassica species have associated pectin and lignin content with shattering. Shattering genotypes of Brassica species showed lesser pectin in the middle lamella at the ripening stages of fruit compared to initial stages of development (Joseffson, 1968). In Brassica campestris, shattering resistance was associated with low levels of lignification of siliqua valve which probably resulted in reduction of forces of dehiscence within the siliqua (Garlicka, 1961). However, Tamaszewski (1964) reported association of shattering resistance in Brassica with thicker valves that were well lignified in the mid valve region and less lignified in the region of the junction between the valves.

No biochemical studies have been undertaken in shattering and shattering resistant genotypes of soybean. Deposition of lignin and dissolution of the wall layer are shown to play a major role in shattering of other crops. Phenylalanine ammonialyase (PAL) is a key enzyme required for secondary metabolism leading to lignin synthesis (Hahlbrock and Scheel, 1979). Since very little information is available on the biochemical changes which precede maturity and shattering of the soybean and since PAL can serve as a good marker for monitoring the synthesis of secondary metabolites, the present study was undertaken. A comparative study of PAL at different developmental stages of the pods in the shattering and shattering resistant genotypes of soybeans has been the objective of the present study.

Materials and Methods

Ten soybean genotypes representing five each from highly shattering (viz. NRC-1, JS-2, Type-49, Lee and CO-1) and five shattering resistant (viz. Bragg, PK-416, Hardee, JS 71-05 and JS 75-46) were planted in a randomized block design with three replications at the Research Farm of National Research Center for Soybean, Indore (India). Each plot consisted of 6 rows 6 m long planted 0.45 m apart with 0.05 m plant spacing within the row. An initial dose of NPK @20:60:20kg ha^{-1} was applied before the planting. All the other recommended agronomic practices were followed throughout the crop growth. Pods were collected at various developmental stages from the fields for enzyme analysis. The developmental stages of the pods were identified according to Fehr and Caviness (1977). The reproductive stages R1 and R2 are based on flowering, R3 and R4 stages on pod development, R5 and R6 on seed development, and R7 and R8 on maturation.

The enzyme analysis was done from R3 stage onwards in the two shattering (JS-2 and NRC-1) and the two shattering resistant (Bragg and PK-416) genotypes. In the rest of the genotypes, analysis was done at the R6 stage. Whole pods (2 cm long) were used at the R3 and R4 stages for the analysis. At later stages from R5 to R8, the pods were dissected and the enzyme was analyzed separately in the dorsal suture, ventral suture and the pod wall. At the R6 stage, a sclerenchymatous layer adhering to the inner wall of the pod is discernible and was removed by the forceps. The PAL was analyzed separately in this layer in addition to the other parts of the pod.

PAL was estimated by the modified method of Zucker (1965). Enzyme was extracted from freshly harvested pods at all the stages of pod development. 500 mg of the material was homogenized in 3 ml of Tris-HCl buffer, pH 8.8, containing mercaptoethanol (0.1%). The extract was squeezed through 4 layers of cheese cloth and centrifuged at 3000g at 4°C for 15 minutes. The supernatant served as the enzyme extract for analysis. The reaction mixture consisted of 2 ml of Tris-HCl buffer, pH 8.8, 0.5 ml of 25 mM phenylalanine, 1 ml of distilled water and 0.5 ml of enzyme extract. In the control, phenylalanine was omitted. The PAL activity was analyzed by monitoring the increase in absorbance at 290 nm for 1 hour and the activity expressed as umoles of t-cinnamic acid/g/h. An increase in absorbance at 290 nm of 0.01 is equal to 3.3 umoles of t-cinnamic acid produced (Zucker, 1968). The data presented is the mean of six replicates and SD was within 5 to 10%.

Results and Discussion

Analysis of PAL activity was done for all the stages of pod development (from R3 to R8) in the genotypes NRC-1, JS-2, Bragg and PK-416. Except for Bragg, the rest of the varieties showed minor differences in their enzyme activity at the R3 stage (Table 1). At the R4 stage (pod 2 cm long), the activity was higher in the shattering genotypes (JS-2, NRC-1) compared to the shattering resistant genotypes (Bragg, PK-416). At the R5 stage (seed initiation) and the R5+ stage (10 days beyond the R5 stage), the PAL activity was higher in the ventral suture of the pod in the two shattering varieties. In the pod wall, the activity was higher in a shattering resistant genotype (Bragg).

At the R6 stage (full seed stage), the enzyme level decreased in sutures and the pod wall in all four genotypes. But in the sclerenchymatous layer which can be separated at the R6

stage, the activity was higher in the shattering genotypes (Table 1).

Because the differences in enzyme activity were found to be more pronounced in the sclerenchymatous layer of the pod wall at R6 stage, the enzyme analysis in this tissue was extended to six more genotypes, three each from shattering and shattering resistant types. A similar difference in the activity was observed in all the genotypes tested (Fig. 1). The activity was 4-5 fold higher in all the shattering genotypes (JS-2, NRC-1, Lee, CO-1, and Type-49) as compared to the shattering resistant genotypes (Bragg, PK-416, Hardee, JS 71-05, JS 75-46). Beyond R6 stage (i.e. R7 and R8) the PAL activity in all parts of the pod in both shattering and shattering resistant genotypes either became very low or no activity was recorded (data not given) which could be due to loss of moisture at R7 and R8 stages.

The data indicate that the PAL activity in general is higher in the shattering genotypes at all the stages. The difference is most pronounced in the sclerenchymatous layer at the R6 stage of the pod compared to the rest of the stages. PAL activity is known to be correlated with the synthesis of various compounds of phenolic metabolism (Edwards et al., 1986; Bevan et al., 1989, Cramer et al., 1989). Concomitant increase in the level of PAL and phenolic compounds has been demonstrated in many plant tissues (Henderson and Friend, 1979; Friend, 1981). Various types of secondary metabolites like flavonoids, lignin, tannin etc. require PAL as an initial enzyme for their synthesis. A high activity of PAL in the sclerenchymatous layer of pods of shattering genotypes, which preceded pod maturity, indicates enhanced level of metabolism leading to synthesis and subsequent deposition of secondary metabolites in this layer. A direct correlation is however yet to be established between accumulation of secondary metabolites and differential shattering behavior.

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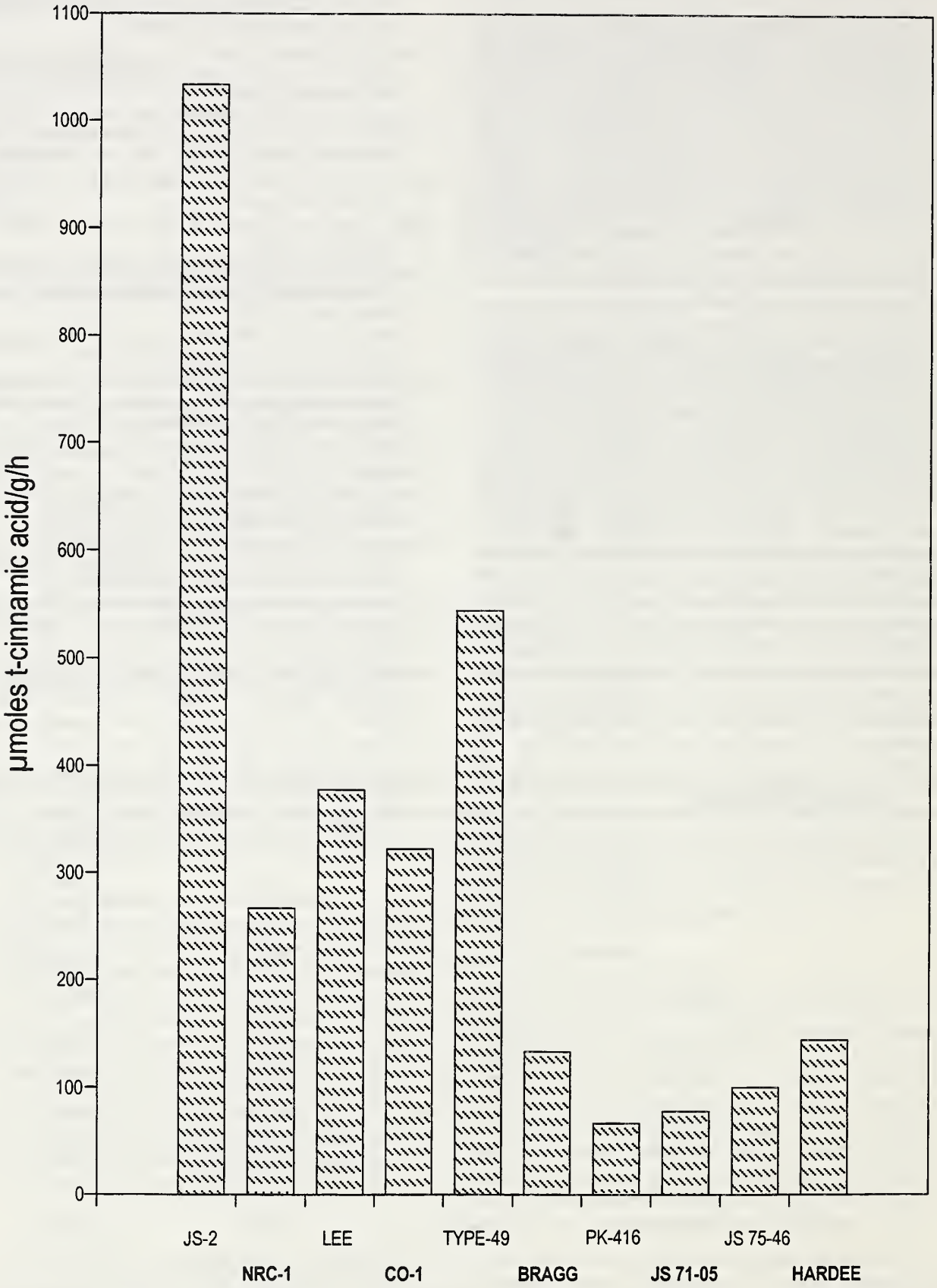
Table 1. Phenylalanine ammonialyase activity (umoles t-cinnamic acid/g/h) in various developmental stages of the pod in shattering (JS-2, NRC-1) and shattering resistant (Bragg, PK-416) varieties.

Stage	Pod region	Varieties			
		JS – 2	NRC - 1	Bragg	PK – 416
R3		340.0 ± 43.0	376.2 ± 45.4	268.4 ± 3.8	390.7 ± 52.4
R4		437.0 ± 42.7	521.4 ± 51.6	136.4 ± 20.7	301.4 ± 40.9
R5	D	747.0 ± 57.9	190.3 ± 18.3	301.4 ± 20.7	603.5 ± 41.1
	V	896.6 ± 69.5	379.5 ± 29.4	170.9 ± 23.2	202.6 ± 17.8
	PW	302.9 ± 23.7	151.8 ± 20.0	236.0 ± 30.1	128.6 ± 03.9
R5±	D	657.0 ± 23.0	446.6 ± 35.0	430.9 ± 50.7	398.3 ± 36.6
	V	904.4 ± 22.7	652.3 ± 22.0	389.5 ± 09.5	219.7 ± 27.6
	PW	359.3 ± 44.5	493.9 ± 51.6	805.4 ± 26.9	168.2 ± 23.9
R6	D	199.1 ± 14.4	71.4 ± 3.4	63.5 ± 6.4	72.0 ± 3.0
	V	125.4 ± 17.4	93.7 ± 9.7	58.0 ± 5.0	69.9 ± 3.3
	PW	67.2 ± 04.3	0	79.2 ± 3.6	62.6 ± 6.7
	SL	1028.0 ± 75.6	267.4 ± 20.2	128.7 ± 10.3	59.8 ± 4.4

D - dorsal suture; V - ventral suture; PW - pod wall; SL - sclerenchymatous layer

Figure 1.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CO-1
TYPE-49
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PK-416
JS 71-05
JS 75-46
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Radiation Induced Variability for Seed Longevity in Soybean Variety NRC-7

Introduction

A major constraint in exploitation of yield potential of soybean in tropics is poor seed longevity. The loss of seed viability in storage is accelerated by high ambient temperature and high relative humidity. These factors interact with incidence of fungal pathogens during maturation period to reduce the quality of maturing seed (Paschal and Ellis 1978). Genotypic differences in seed storability have been reported, bolder seed size is reported to be associated with poor seed viability (Wien & Kueneman 1981). NRC-7, a recently released variety of soybean in M. P. has high yield, early maturity and resistance/tolerance to insect pests but suffers from poor seed longevity. The present study was aimed at creating variability for seed longevity through induced mutations and selection of desirable mutants.

Materials and Methods

Dry seeds of soybean variety NRC-7 were exposed to gamma rays (15 KR). M1 & M2 generations were raised. Seeds of 2600 M2 plants were examined for better appearance and smaller size. The selected 410 plants were grown in progeny rows in rainy season of 1995 in augmented design with four checks. Uniform rows were bulked. Resulting M4 seeds were screened for seed longevity through accelerated aging. 100 seeds in 4 replications were kept at 42^0 C and 100% RH for 24 hours after treating with thiram. This method was a modification of procedure given by Byrd and Delouche (1971), as the period was reduced from 48 to 24 hours. It was found by standardization that 24 hrs were sufficient to bring out genotypic difference. Moreover exposure for 48 hours was found to be too severe resulting in no germination in NRC-7. The mutant lines with high germination after accelerated aging were tested for field emergence in rainy season of 1996 after ambient storage of 8 months. 100 seeds were sown in a 5m now in three replications in completely randomized block design. The seeds of same lots were tested for laboratory germination after 18 months ambient storage. The data was analysed as per standard procedures.

Results and Discussion

Highly significant differences were observed among M3 families for Germination after accelerated aging and seed size. 34 M3 families were having germination in range of 60-83% after accelerated aging. Out of these, 16 families showed superior agronomic performance. All these families show marked improvement in germination over parent NRC-7 (27%) (Table 1). The germination was comparable to best check Kalitur (84%) an indigenous black seeded cultivar known for exceptionally good seed longevity. Field emergence of the mutant lines after 8 months' ambient storage also showed significant superiority over NRC-7. Bhatnagar and Karmakar (1995) have emphasized that varieties for tropics should have more than 70% germination after 8-9 months ambient storage. In this experiment most of the lines had above 70% germination. Field emergence ranged from 29% (NRC-7) to 82% (119-7). A positive linear correlation (r = 0.49) was observed between germination after accelerated aging and field emergence. Delouche & Baskin (1973) found that germination after accelerated aging and period of storage were closely associated. The reduction in viability was drastic after 18 months storage, the mean germination declined by 33% during 10 months. Only four lines showed germination above 50%. Three lines 158-15, 262.5 and 387-1 were statistically at par with best check Kalitur and one line 150-3 with JS 335 & Punjab 1.

The mutant progenies possessed bold seeds comparable to parent NRC-7 except for one line 119-7. Bolder seed size is associated with poor seed longevity (Bhatnagar & Karmakar 1995). Germination after accelerated aging and field emergence after 8 months storage showed no correlation with seed size. Germination after 18 months storage was, however, negatively associated (r = -0.68) with seed size. Wien & Kueneman (1981) also observed a negative correlation between storability and seed size.

Four mutant lines viz. 150-3, 158-15, 262-5, and 387-1 were found to possess good germinability after 18 months storage at ambient temperature. These lines will be further tested for yield in multiplication trials. They can also be used as donors for good seed longevity.

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Table 1. Seed longevity and seed size of M4 progenies of NRC 7

Mutant Progeny	Germination after accelerated aging (%)	Field emergence after 8 months ambient storage (%)	Germination after 18 months ambient storage (%)	Seed size (g/100)
48-4	63	78	31	16.4
109-17	71	72	41	17.0
117-7	63	71	41	19.2
119-7	82	82	45	15.3
122.5	68	74	47	18.6
127.1	76	65	30	19.6
148-10	76	72	36	17.2
150-3	80	79	52	17.3
150-4	86	72	36	17.4
158-15	61	75	67	16.5
169-1	76	72	23	18.5
186-2	63	69	39	18.2
195-1	82	40	26	17.1
262-5	86	73	60	16.5
387-1	72	78	64	17.2
NRC-7	27	29	36	18.1
JS 335	64	70	70	11.4
Kalitur	84	82	78	12.1
Punjab-1	53	73	67	9.8
Mean	**70.8**	**68.8**	**45.5**	**16.5**
C.D. (0.05)	**22.0**	**13.0**	**19.0**	**2.4**
C.V. (%)	**8.5**	**14.0**	**27.0**	**7.1**

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Regulation of PAL Activity and Pod Shattering in Soybean Varieties after Treatment with t-Cinnamic Acid and Cobalt Chloride

Introduction

Our earlier results have established that the shattering varieties of soybean have a high activity of phenylalanine ammonialyase (PAL) compared to the shattering resistant varieties especially in the sclerenchymatous layer of the pods at R6 stage. PAL is the key enzyme for secondary metabolism in plants and modulation of its activity affects all products of phenylpropanoid pathway like flavonoids, tannins, lignin etc. The present investigation has been aimed at answering whether regulation of PAL activity by known inhibitors of the enzyme affects shattering of the pods.

Trans-cinnamic acid inhibits the activity of PAL competitively by feedback regulation (Camm and Towers, 1973). Cobalt chloride is the other known inhibitor of PAL which inhibits the activity by binding to enzyme-substrate complex (Dubey et al., 1993). These two inhibitors have been used in the present study.

Materials and Methods

Two shattering varieties of soybean JS-2 and NRC-1 were grown in pots maintaining three plants per variety in each pot in three replications. The plants were sprayed with trans-cinnamic acid (10^{-4} and 10^{-3} M) solutions made in distilled water using a hand sprayer. The whole plants were sprayed from R5 (seed initiation) stage up to R8 (harvest maturity) stage. The stages were identified according to Fehr and Caviness (1977). Spraying was done at an interval of 5 days and the plants received a total of 8 sprays between R5 and R8 stages, control plants were maintained without treatment with the chemicals.

PAL was analyzed in both the varieties before spraying the chemicals at R5 stage. After the treatment PAL was analyzed at R6 stage. Analysis was done separately for different parts of the pod. PAL was estimated by the modified method of Zucker (1965) as described before.

Percent shattering of pods was calculated by counting the shattered vis-a-vis total pods on the seventh day after harvest maturity (about 13% seed moisture).

Results and Discussion

Before the treatment with inhibitors, PAL activity at R5 stage was high in the dorsal and ventral sutures of both the varieties (Table 1). When the pods reached R6 stage, there was a decline in the activity in both the dorsal and the ventral sutures; in contrast to this the sclerenchymatous layer showed a high activity of PAL. Both t-cinnamic acid and $CoCl_2$ inhibited the activity of PAL significantly at R6 stage compared to the controls. Inhibitors reduced the activity of PAL by over ca 50% in JS-2 though they were less effective on NRC-1 (Table 1). In JS-2 there was no significant effect of inhibitors on the activity of PAL in the dorsal suture while the activity was inhibited by over ca 50% in the ventral suture. In NRC-1 the inhibitors prevented the decline of PAL activity in dorsal and ventral sutures from R5 to R6 stage.

The effect of inhibitors on the pod shattering in the varieties was not of the same magnitude as that of inhibition of enzyme activity (Table 2, 3). t-cinnamic acid did not alter the percentage of shattering of pods in JS-2 and in NRC-1 the shattering of pods was reduced by a maximum of 10% at 10^{-3} M (Table 3). $CoCl_2$ was more effective in reducing the percentage of shattering in both the varieties. A maximum reduction of 25.2% (at 100 µM) in JS-2 and 19.5% (at 10 µM) in NRC-1 was recorded with $CoCl_2$ treatment.

The inhibitory effect of t-cinnamic acid and $CoCl_2$ on the PAL activity was much higher than their influence on the shattering behavior of the varieties as established by our results. An exact quantitative correlation between the extent of inhibition of PAL activity with the extent of prevention of shattering was not observed in any of the varieties. Results indicate that although a higher activity of PAL exists in the sclerenchymatous layer of the pods of the shattering varieties, PAL may not be the sole contributor for the shattering behavior of the variety. However, $CoCl_2$ emerges as a promising chemical with the recorded prevention of 20-25% of shattering in the varieties tested and merits further field trials. Both $CoCl_2$ and t-cinnamic acid had no detrimental effect on the vegetative growth of the plants, and development of pods and seeds (data not given).

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Table 1. Effect of exogenous application of t-cinnamic acid and $CoCl_2$ on PAL activity at R6 stage in JS-2 and NRC-1 varieties of soybean (±SD).

Stage	Pod region	JS – 2	NRC – 1
R5 stage (before treatment)	D	782.5 ± 58.0	699.6 ± 12.3
	V	831.0 ± 75.5	723.3 ± 50.7
	PW	366.3 ± 22.7	498.3 ± 39.2
R6 stage (after treatment)	D	165.0 ± 5.5	99.0 ± 5.0
	V	474.1 ± 15.9	85.8 ± 7.5
	SL	1026.3 ± 96.9	580.8 ± 41.3
t-cinnamic acid (10^{-4} M)	D	166.0 ± 7.0	245.3 ± 24.5
	V	159.5 ± 20.6	161.7 ± 20.0
	SL	427.6 ± 25.4	389.4 ± 25.0
t-cinnamic acid (10^{-3} M)	D	166.1 ± 10.4	188.7 ± 22.7
	V	161.7 ± 15.0	150.5 ± 18.4
	SL	502.7 ± 60.0	460.3 ± 9.7
$CoCl_2$ (10 µM)	D	135.3 ± 9.9	134.2 ± 8.2
	V	123.2 ± 4.9	244.2 ± 23.0
	SL	343.2 ± 29.2	483.0 ± 29.0
$CoCl_2$ (100 µM)	D	165.0 ± 13.0	260.0 ± 30.7
	V	154.0 ± 18.0	148.8 ± 18.0
	SL	405.9 ± 18.9	267.3 ± 9.9

D = dorsal suture;
V = ventral suture;
PW = pod wall;
SL = sclerenchymatous layer.

Table 2. Percent shattering of pods in the varieties JS-2 and NRC-1 after treatment with t-cinnamic acid and $CoCl_2$.

Treatment	JS – 2	NRC – 1
0	55.7 ± 1.0	69.3 ± 6.6
t-cinnamic acid (10^{-4} M)	55.0 ± 5.0	65.5 ± 4.5
t-cinnamic acid (10^{-3} M)	64.0 ± 5.8	62.0 ± 4.0
$CoCl_2$ (10 µM)	50.2 ± 3.68	55.3 ± 1.8
$CoCl_2$ (100 µM)	41.7 ± 6.8	61.8 ± 4.8

Table 3. Percent inhibition of PAL in sclerenchymatous layer (at R6 stage) and percent prevention of shattering of pods in JS-2 and NRC-1 after treatment with t-cinnamic acid and $CoCl_2$.

Treatment		JS – 2	NRC - 1
t-cinnamic acid (10^{-4} M)	E	58.4	33.0
	P	0	5.4
t-cinnamic acid (10^{-3} M)	E	51.1	20.8
	P	0	10.5
$CoCl_2$ (10 µM)	E	66.6	16.9
	P	10.0	19.5
$CoCl_2$ (100 µM)	E	60.5	54.1
	P	25.2	11.0

E = enzyme
P = pod shattering.

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Seed Size Parameters of Soybean Varieties of Commercial Importance

For production and processing of quality seed knowledge of seed size parameters of different soybean varieties is important. In India, out of 62 varieties of soybean released so far, only 21 are currently in seed production chain. Reports on various seed size parameters of these varieties are not available. In this study, the seed size parameters of different varieties and the influence of sowing dates on seed weight are presented.

Material and Methods

Pure seeds harvested by bulking true-to-type plant-to-progeny rows for each of the 21 varieties in rainy season of 1997 were used for measurements of seed size. Observations were recorded for length, breadth and thickness of seeds and 100 seed weight. Correlation among these characters were calculated.

In another experiment conducted in rainy season of 1996, 16 varieties were sown in row plots on 6 different dates at the interval of 10 days i.e. on June 6, June 16, June 26, July 6, July 16 and July 26. Seed size of the varieties harvested from each of these sowing dates were measured in terms of 100 seed weight (g).

Results and Discussion

Seed length, breadth and thickness varied between 0.57 to 0.70 cm, 0.51 to 0.65 cm and 0.40 to 0.54 cm respectively among the varieties (Table 1). The length to breadth ratio of seeds varied between 1.00 to 1.30. Breadth and thickness of seeds showed strong positive correlation ($r = 0.8355$; $P = 0.01$) whereas length and breadth of the seeds showed low positive correlation ($r = 0.3704$; $P = 0.05$). No correlation was observed between length and thickness of seeds. In most of the varieties, seeds are elliptical in shape (length to breadth ratio is greater than unity) except Monetta, JS 71-05, NRC 12, Pusa 20 and JS 335 where seeds are roundish in shape. 100 seed weight of the varieties varied between 8.0 to 14.0 gm. Seed length, breadth and thickness showed strong and positive correlation with seed weight ($r = 0.6451, 0.7472, 0.7219$ respectively; $P = 0.01$). Seeds with high 100 seed weight were bigger in seed size. Among seed size parameters, 100 seed weight is the easiest to measure.

Mean seed weight of varieties over 6 sowing dates varied from 7 g (for Kalitur) to 16 g (for Pusa 24). Mean seed weight under different sowing dates over 16 varieties varied from 11.08 g (July 26 sowing) to 12.30 g (July 6 sowing). Some varieties like JS 80-21, Pi< 327, Pusa 20, Punjab I and JS 335 showed less variation in seed weight over sowing dates while some varieties Pusa 24, Pusa 22 and PK 416 showed relatively more variation.

This study has indicated the presence of considerable variation in seed shape, size and weight parameters among the varieties currently in seed production chain and seed weight of these varieties is to some extent influenced by the sowing dates.

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Table 1. Seed dimension and 100 seed weight of 21 soybean varieties currently under seed chain.

Variety	Seed Dimension				
	Length (cm)	Breadth (cm)	Thickness (cm)	Length to Breadth ratio (L:B)	100 seed weight (g)
NRC 7	0.70 ± 0.041	0.62 ± 0.031	0.54 ± 0.027	1.13	14.0
MACS 124	0.70 ± 0.038	0.54 ± 0.029	0.41 ± 0.031	1.30	10.0
NRC 12	0.68 ± 0.041	0.64 ± 0.045	0.52 ± 0.055	1.06	13.0
Pusa 20	0.67 ± 0.041	0.62 ± 0.034	0.52 ± 0.039	1.08	13.5
Pusa 16	0.67 ± 0.026	0.57 ± 0.021	0.48 ± 0.025	1.17	11.0
PK 472	0.67 ± 0.034	0.56 ± 0.035	0.42 ± 0.025	1.20	12.0
JS 71-05	0.66 ± 0.059	0.62 ± 0.017	0.53 ± 0.028	1.06	12.7
PK 416	0.66 ± 0.034	0.54 ± 0.020	0.46 ± 0.030	1.22	11.5
Monetta	0.65 ± 0.043	0.65 ± 0.042	0.51 ± 0.041	1.00	11.3
NRC 2	0.65 ± 0.048	0.56 ± 0.037	0.48 ± 0.038	1.16	9.0
PK 1029	0.65 ± 0.036	0.55 ± 0.024	0.44 ± 0.034	1.18	10.5
MACS 13	0.64 ± 0.030	0.58 ± 0.041	0.45 ± 0.029	1.10	9.5
PK 262	0.64 ± 0.038	0.57 ± 0.028	0.45 ± 0.031	1.12	11.0
PK 564	0.64 ± 0.056	0.56 ± 0.040	0.42 ± 0.046	1.14	10.5
PK 1042	0.64 ± 0.037	0.56 ± 0.025	0.46 ± 0.039	1.14	11.0
PK 471	0.64 ± 0.044	0.55 ± 0.024	0.43 ± 0.034	1.16	10.0
PK 1024	0.62 ± 0.043	0.57 ± 0.025	0.49 ± 0.035	1.09	11.0
JS 335	0.62 ± 0.044	0.57 ± 0.030	0.48 ± 0.030	1.08	12.0
MACS 58	0.62 ± 0.049	0.53 ± 0.045	0.40 ± 0.056	1.17	9.3
MACS 57	0.62 ± 0.026	0.51 ± 0.027	0.44 ± 0.036	1.21	8.7
PK 308	0.57 ± 0.032	0.53 ± 0.034	0.43 ± 0.039	1.07	8.0
Mean	0.64 ± 0.029	0.56 ± 0.037	0.46 ± 0.042	1.13 ± 0.01	10.9 ± 0.35
Range	0.57 to 0.70	0.51 ± 0.65	0.40 ± 0.54	1.00 to 1.30	8.0 to 14.0

Table 2. Influence of different sowing dates on seed size (100 seed weight in gm) of soybean.

Variety	Sowing dates in 1996						Mean
	June 6	June 16	June 26	July 6	July 16	July 26	
JS 71-05	10.7	11.0	12.7	11.7	12.4	10.3	11.30 ± 0.39
Punjab 1	9.3	9.0	9.3	9.4	8.0	8.0	8.90 ± 0.27
JS 335	11.3	10.0	11.7	12.0	11.0	10.7	11.11 ± 0.29
NRC 2	11.7	10.4	10.7	12.3	11.7	10.7	11.20 ± 0.31
PK 472	11.7	13.7	11.7	12.7	13.7	12.7	12.70 ± 0.37
Bragg	10.7	10.3	9.0	11.7	11.7	10.3	10.60 ± 0.41
JS 80-21	10.0	10.0	10.0	10.0	9.3	9.3	9.80 ± 0.15
PK 416	9.3	10.3	13.3	13.0	12.7	11.7	11.71 ± 0.66
Pusa 20	15.3	14.7	13.7	14.7	14.0	14.7	14.50 ± 0.23
Pusa 22	11.7	9.0	9.0	11.3	10.0	13.7	10.80 ± 0.74
Shivalik	14.0	13.7	14.0	12.7	12.7	12.0	13.20 ± 0.34
PK 471	11.7	12.3	13.0	14.7	14.0	13.0	13.11 ± 0.45
Kalitur	6.5	-	8.0	6.0	7.7	7.0	7.00 ± 0.37
PK 262	13.3	14.0	12.0	14.7	12.7	-	13.30 ± 0.47
Pusa 24	12.0	15.7	18.0	17.7	16.7	-	16.00 ± 0.08
PK 327	10.3	10.0	10.3	-	10.0	11.0	10.33 ± 0.18
Mean	11.22 ± 0.51	11.61 ± 0.57	11.65 ± 0.63	12.30 ± 0.70	11.77 ± 0.59	11.08 ± 0.57	11.63

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Stability Analysis of Soybean Varieties under Different Plant Densities and Growing Conditions

Introduction

In India, soybean *[Glycine max* (L.) Merrill] has become one of the important oilseed crops covering an area of about 5 million hectares with a productivity of about 1 ton/ha. It is cultivated in about 8 states of India under varied climatic and growing conditions viz. rainfed and irrigated. Earlier work on stability analysis of soybean varieties has been mainly confined to their stability under different locations (Angeles, 1987; Bhatnagar & Tiwari, 1989; Taware, *et al.*, 1991 and Tiwari, *et al.*, 1994) and seasons (Patil *et a/.*, 1988; Clark & Snyder, 1989; Raut, *et al.*, 1990; Raut, *et al.*, 1992; Taware, *et al.*, 1994; Raut, *et a/.*, 1997 and Mebrahta & Elmi, 1997). However, meagre work has been reported regarding their stability under different plant densities and growing conditions. Since the crop plants perform differently under varied plant population levels and irrigation conditions, present work has been undertaken to study stability of some promising soybean varieties under such conditions.

Materials and Methods

Eight promising varieties of soybean were planted under 0.2, 0.4 and 0.6 million plant population levels (m/ha) under rainfed and irrigated conditions in rainy seasons of two successive years (1993, 1994). Thus twelve environments (3x2x2) were created to test the phenotypic stability of varieties under study. Each variety was grown in 8 rows of 6m length with three replications. Plant population levels were maintained by using appropriate seed rate. Data on days to flower, days to maturity, plant height (cm), pods/plant, 100 seed weight (g) and seed yield (g/net plot) were recorded for all the treatments. Data for seed yield was converted into kg/ha and used for further statistical analysis. Data were analysed for analysis of variance by standard methods. Mean values were used for stability analysis and pooled analysis of variance, and stability parameters were computed by using the model of Eberhart and Russell (1966).

Results and Discussion

Analysis of variance of mean data (Table 1) indicated significant differences for all the characters among the linear component of variation in environments. Mean squares for varieties were significantly different for all the characters except for pods/plant indicating variability among varieties under test environments. However, interaction between varieties and environments was significant only for days to flower, days to maturity, pods/plant and 100 seed weight.

Mean ($\bar{X}$), regression coefficients (b_i) and values for deviation from regression (S^2d) of eight varieties for different characters are presented in Table 2. Mean performance of eight varieties for seed yield (kg/ha) indicated MACS 63 to be the highest yielding variety (2032 kg/ha). MACS 124 and MACS 13 gave seed yield of 1959 and 1924 kg/ha, respectively, which were at par with MACS 63. These two varieties were midlate in maturity while MACS 63 was significantly earlier. UGM 30 recorded maximum plant height while PK 472 and Bragg were with short stature. MACS 63 had the highest pod number per plant (34.8), however, except for Bragg (29.8) the remaining varieties were at par with it. MACS 124, MACS 13, PK 472 and Bragg showed significantly higher 100 seed weight than other varieties and were at par with each other.

On the basis of values of regression coefficient, except for PK 472 and KB 60, all the varieties showed good stability for seed yield (kg/ha). MACS 63 and MACS 13 showed minimum deviation of regression coefficient from unity (-O.02 and -0.14, respectively). However, MACS 63 recorded significant deviation from regression. On the basis of three stability parameters MACS 13, MACS 63 and MACS 124 had more than average stability under different plant population levels and growing conditions. These three varieties also showed more than average stability for other characters. Taware *et al.* (1994) have reported MACS 63 and MACS 124 as stable varieties for seed yield for rainy and summer seasons. Recently, Raut *et al.* (1997) found MACS 124 as stable genotype for high yield in rainy season.

Mean performance of eight varieties for seed yield (kg/ha) under different environments and environmental indices along with mean and CD values are given in Table-3. It can be seen that, in general, yield levels were in the order of 0.2<0.4<0.6 m/ha plant population level under both irrigated and rainfed conditions. Yield levels were higher under irrigated conditions than under rainfed condition.

It is significant to note that MACS 13 and MACS 124 varieties, identified as stable varieties in the present studies, have been released for cultivation in Central and Southern India, respectively. In addition, MACS 63 variety showing maximum mean performance for yield, more than average stability as also early maturity in the present investigation will be a useful genotype for breeding for earliness and high yield.

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Table 1. Analysis of variance (mean squares)

Source	D. F.	Days to flower	Days to maturity	Plant height(cm)	Pods/plant	100-seed weight(g)	Yield(kg/ha)
Varieties	7	241.40**	210.52**	2549.82**	24.11	22.80**	568923.86**
Environment + (Varieties x Environments)	88	7.07**	16.85**	80.87**	55.51**	2.43**	171637.50**
Environment (Linear)	1	538.03**	1298.55**	4716.76**	3593.59**	163.99**	9236578.44**
Varieties x Environment (linear)	7	4.04**	14.20**	34.87	35.27*	2.85**	121762.83
Pooled deviation	80	0.70**	1.06	26.94	13.05	0.37	62689.77**
Pooled error	192	0.24	0.94	20.95	12.77	0.35	11045.13

*, ** Significant at 5% and 1%, respectively.

Table 2. Stability parameters for different characters in soybean

Variety	Days to flower			Days to maturity			Plant height (cm)			Pods/plant			100-seed weight (g)			Yield (kg/ha)		
	$\bar{X}$	b_i	S^2d	$\bar{X}$	b_i	S^2d	$\bar{X}$	b_i	S^2d	$\bar{X}$	b_i	S^2d	$\bar{X}$	b_i	S^2d	$\bar{X}$	b_i	S^2d
MACS 124	45.06	0.72*	0.79**	93.33	0.88	0.45	63.83	0.91	24.93**	33.26	0.66*	-4.22	13.74	1.23	-0.09	1958.75	1.31	27331.87**
MACS 63	44.64	1.13*	-0.03	85.94	1.15	-0.26	57.88	1.02	38.84**	34.79	1.18	2.99	10.38	1.16	-0.13	2032.16	0.98	63077.68**
KB 60	46.72	0.89	0.05	98.03	0.52	0.11	52.69	1.29*	-9.24	32.97	1.15	-3.12	12.32	0.92	0.07	1740.14	1.36*	18246.55**
UGM 30	50.28	1.45**	0.57**	96.67	1.01	1.67**	77.02	1.08	14.05	32.14	0.97	1.86	10.17	0.24	-0.17	1481.28	0.50	108367.96**
PK 472	43.75	1.23	1.08**	89.64	1.45	-0.13	34.14	0.60**	-15.46	31.97	1.18	-8.29	13.31	1.54	-0.02	1730.45	1.40**	4601.39
JS 81714	40.72	0.85	0.48**	91.31	1.06	-0.85	44.37	1.34	1.04	31.98	0.95	1.43	12.46	1.09	-0.03	1614.03	0.80	138446.27**
Bragg	35.06	0.82**	-0.22	90.17	1.22	0.84*	35.23	0.84	3.88	29.79	0.55**	-0.14	13.06	0.95	0.46*	1453.74	0.79	57885.57**
MACS 13	45.31	0.91	0.91	96.56	0.71	-0.83	46.46	0.93	-10.08	32.16	1.37	11.74*	13.48	0.87	0..05	1923.99	0.86	-4800.17

*, ** Significant at 5% and 1%, respectively.

Table 3. Mean performance of soybean varieties for yield (kg/ha) and environmental indices (I_j) of different environments

Variety	Year (1993)						Year (1994)						Mean	Rank
	Irrigated			Rainfed			Irrigated			Rainfed				
	0.2 m/ha	0.4 m/ha	0.6 m/ha	0.2 m/ha	0.4 m/ha	0.6 m/ha	0.2 m/ha	0.4 m/ha	0.6 m/ha	0.2 m/ha	0.4 m/ha	0.6 m/ha		
MACS 124	2259	2498	2629	2002	2150	2450	1618	1950	1891	1196	1360	1503	1959	2
MACS 63	2235	2407	2450	1883	1963	1915	2265	2603	2279	1334	1572	1480	2032	1
KB 60	1564	2275	2282	1647	1855	1873	1438	2043	2465	1169	1153	1118	1740	4
UGM 30	1765	1733	1479	1048	1200	1199	1508	2061	2139	1274	1252	1119	1481	7
PK 472	1846	2341	2424	1675	2027	2020	1135	1854	1934	1081	1293	1135	1730	5
JS 81714	993	1602	1955	1937	2265	2276	916	1445	1760	1173	1445	1602	1614	6
Bragg	1265	1368	1835	1794	1685	2189	1126	1276	1552	1009	1165	1181	1454	8
MACS 13	2104	2235	2331	1884	2019	2121	1604	2105	2031	1482	1659	1513	1924	3
Mean	1754	2057	2173	1734	1895	2005	1451	1917	2006	1215	1362	1331	-	-
CD at 5%	277	393	201	256	250	343	201	235	398	2400	221	174	-	-
I_j	12	315	431	-8	154	264	-291	175	265	-527	-380	-411	-	-

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A Gene for Insensitivity of Flowering to Incandescent Long Daylength (ILD) Is Located in the Linkage Group 4

Introduction

Six loci have been reported to control time of flowering and maturity in soybean ; *E1* and *E2* (Bernard, 1971), *E3* (Buzzell 1971), *E4* (Buzzell and Voldeng, 1980), *E5* (McBlain and Bernard 1987) and *J* (Ray et al. 1995). Of these, *E1* and *E3* are assigned to the linkage group 1 (Weiss 1970) and 5 (Cober and Voldeng, 1996), respectively. *E2* is linked to *Pgm1* (phosphoglucomutase isozyme-1) and forms a new unnamed linkage group (Akkaya et al. 1995). However, the other genes remain to be assigned to the specific linkage groups. We have been studying the genetic basis of early-maturing habits in land races and local varieties of northern Japan. In the course of study, we found that a gene for insensitivity of flowering to incandescent long daylength (ILD) was linked to *Ln* (leaf shape) and *Enp* (endopeptidase isozyme), members of the linkage group 4.

Materials and methods

A cross was made between early-maturing determinate varieties, "Ohyachi 2 go" (insensitive to ILD) and "Tokachinagaha" (sensitive to ILD), in 1989. F1 plants were grown in a field to produce F2 seed in 1990. The F2 seed was germinated in petri dishs in a growth cabinet of 25 degree C. The three-day old germinated seed was transplanted into paper pots on 20 May, 1991, after a small piece of cotyledon was sampled for isozyme and seed protein analysis. Two weeks later, the resultant seedlings were divided into two groups, each of which was transplanted in experimental fields with and without a facility for extending daylength. The ILD was provided by extending the natural photoperiod to 20 h by means of 500-W incandescent lamps placed 2 m above the soil surface at intervals of 4 m. Date of the first appearance of an open flower (R1) was recorded every other day. A progeny test was performed under ILD in 1992 and 1993 using 157 F3 families derived from F2 plants grown in ND.

Linkage relationship was tested between a gene for insensitivity to ILD and a number of markers segregating in the progeny ; *Ln, Aco1, Dia1, Enp, Idh2* and *Ti*. Analysis for isozymes and seed proteins followed the methods of Abe et al. (1992) and Hymowitz and Hadley (1972), respectively.

Results and Discussion

"Ohyachi 2 go", a pure-line selection from a landrace "Ohyachi", had been widely cultivated in Hokkaido before 1950's because of its high adaptability. Two cultivars, "Kitamishiro" and "Isuzu", were developed from the cross between "Ohyachi 2 go" and "Tokachinagaha" in 1950's. "Kitamishiro" and "Isuzu" were insensitive to ILD as "Ohyachi 2 go", the former of which was determined to have the genotype *Dt1Dt1E1E1e3e3e4e4* by Saindon et al. (1990).

Average and range of the date of R1 for "Ohyachi 2 go" were almost similar between ILD and natural daylength (ND), whereas the date of R1 of "Tokachinagaha" retarded about two weeks under ILD compared with ND (Figure 1). F2 population segregated in a bimodal pattern within two discrete classes in both daylength conditions, although variation was also observed within classes. Based on the date of R1, the F2 plants were classified into 56 early and 188 late plants in ND, 64 early and 178 late plants in ILD. Segregation in both daylengths thus showed a good fit to a 1:3 (early/late) phenotypic ratio expected for a single gene control with the early maturity being recessive (Chi-square value = 0.546, $0.25<p<0.50$ in ND, Chi-square value = 0.270, $0.50<p<0.75$ in ILD). A reseccive gene (*e*(t)) thus was involved in the early flowering of "Ohyachi 2 go" associated with the ILD insensitivity. However, a few plants in the early class flowered earlier than "Ohyachi 2 go", and in the late class there were plants which flowered later than "Tokachinagaha". The transgressive segregation may suggest that another gene hypostatic to the e(t) was also involved in the control of flowering in the parents used.

Response to ILD was also evaluated in the 157 F3 families according to the same method to the experiment of 1992. Based on segregation patterns of the insensitivity to ILD, the families were classified into 43 families homozygous for the insensitivity (early-flowering), 47 families homozygous for the sensitivity (late-flowering), and 67 families segregating for the both. The segregation ratio for the three classes fitted to a 1:2:1 ratio expected for a monogenic inheritance (Chi-square value = 3.573, $0.10<p<0.25$) to confirm the results obtained in F2.

A linkage test revealed that *Ln* and *Enp*, members of the linkage group 4, showed linkage with the gene e(t). The recombination value was estimated with F2 and F3 data as 8.3 % for e(t) vs. *Enp* and 13.8 % for e(t) vs. *Ln*, according to the maximun likelihood method of Allard (1950). The recombination value between *Ln* and *Enp* was calculated as 8.6 %, approximating to a value of 9.38 % reported by Muehlbauer et al. (1989). The order of the three genes therefore was estimated as *Ln-Enp-e*(t).

Buzzell and Voldeng (1980) found that two genes, *E3* and *E4*, were involved in the response of flowering to ILD. Of the two genes, *E3* is linked to *Dt1* (determinate stem) with a recombination value of 27.5 % and is assigned to the linkage group 5 (Cober and Voldeng, 1996). The gene e(t) in the linkage group 4 thus is not e3, but rather seems to be *e4*. A study with a tester is under way to confirm allelism between *e4* and *e*(t).

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Table 1. Linkage test between a gene for ILD-insensitivity (e*(t)*) and marker loci (*Enp* and *Ln*) of Linkage group 4 in the cross between "Ohyachi 2 go" and "Tokachinagaha".

Linkage pair	No.of plants									n	Chi-square for linkage	Recombination value (%)
	AA			Aa			aa					
A vs. B	BB	Bb	bb	BB	Bb	bb	BB	Bb	bb			
Enp-e(t)	2	5	37	7	60	5	38	2	1	157	222.4 ***	8.3 (1.6)
Ln-e(t)	2	11	36	11	48	7	34	8	0	157	161.9 ***	13.8 (2.1)
Enp-Ln	41	3	0	7	57	8	1	6	34	157	217.3 ***	8.6 (1.7)

Values within parenthesis show standard errors.

*** ; Significant at 0.1 % level.

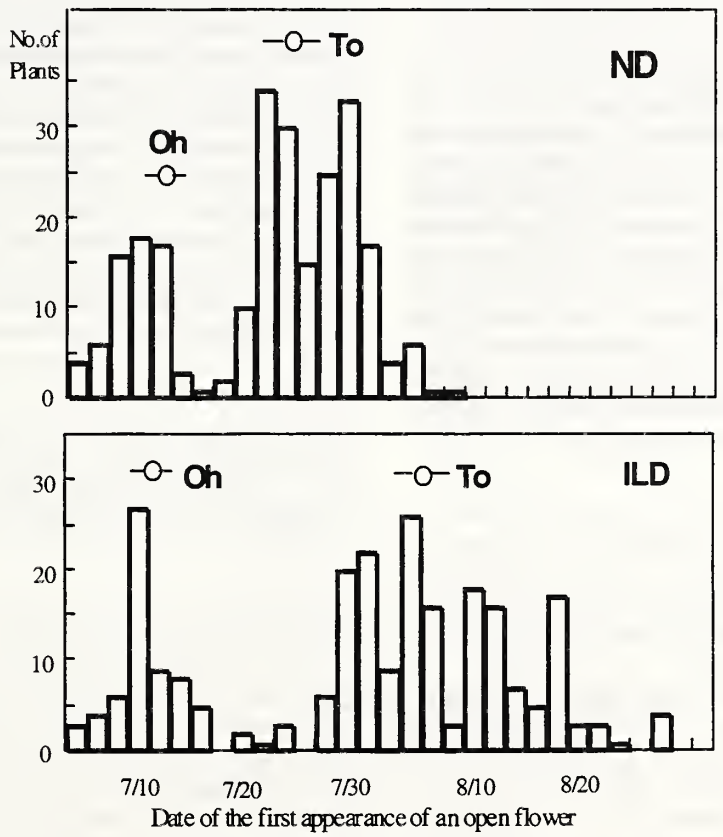

Fig. 1. Frequency distribution of parents and F2 progeny on the date of the first appearance of an open flower in "Ohyachi 2 go"(Oh) and "Tokachinagaha" (To) in natural daylength (ND) and incandescent long daylength (ILD).

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A New Gene for Insensitivity of Flowering to Incandescent Long Daylength (ILD)

Introduction

Insensitivity of flowering to long daylengths is an important character in adaptation of soybean to high latitudinal regions. Of six loci reported to control time of flowering and maturity in soybean, *E3* and *E4* are known to be involved in the response of flowering to long daylength ; *e3* singly controls the insensitivity to fluorescent long daylength (FLD) by extending natural daylength to 20 h using cool white fluorescent lamps, whereas *e4* combines with *e3* to control the insensitivity to incandescent long daylength (ILD) by extending natural daylength to 20 h using incandescent lamps (Buzzell 1971, Buzzell and Voldeng 1980). A new gene affecting the insensitivity to ILD was found in the progeny of a cross between early-maturing land races of Hokkaido, Japan. In this report we present the data on the segregation of the insensitivity to ILD.

Materials and methods

A cross was made between "Miharu Daizu" and "Sakamoto Wase" in 1991. A F1 plant of the cross was grown in the winter in a green house to produce the F2 seed. The F2 seed was sown in paper pots on 2 June, 1992. Two weeks later, the resultant seedlings were transplanted in an experimental field with a facility for extending daylength. The ILD sensitivity of F2 plants was evaluated under long daylength provided by extending the natural photoperiod to 20 h by means of 500-W incandescent lamps placed 2 m above the soil surface at intervals of 4 m. Date of the first appearance of an open flower (R1) was recorded every other day till 19 August when the ILD treatment was ended to promote the sensitive plants to produce F3 seed.

Results and Discussion

Both of "Miharu Daizu" and "Sakamoto Wase" are insensitive to ILD, and mature without any retardation under ILD compared with natural daylength (ND). When seeded under ND of Sapporo in the early June, they mature in approximately 100 days (Sakamoto Wase) to 110 days (Miharu Daizu). However, the two land races are different in genotypes at a number of isozyme loci and RFLP profiles for chloroplast and mitochondrial DNAs, suggesting their independent origins (unpublished data). A crossing experiment thus was conducted to determine allelism between genes involved in the insensitivity of the two land races.

"Miharu Daizu" and "Sakamoto Wase", on the average, flowered in 36 d and 38 d after transplanting, respectively, when grown under ILD (Table 1). The F2 progeny, on the other hand, showed a wide variation from plants which flowered earlier than both parents to those which produced no flower buds till the end of the experiment.

Buzzell and Voldeng (1980) found that a double recessive homozygote (*e3e3e4e4*) at *E3* and *E4* loci conditioned the insensitivity of flowering to ILD. If the insensitivity of both land races to ILD is controlled by *e3e3e4e4* as indicated by Buzzell and Voldeng (1980), all of the segregants of the cross are expected to be insensitive to ILD. The result obtained was evidently not consistent with the expectation. The segregation of late-flowering or non-flowering plants indicates that the insensitivity of "Miharu Daizu" and "Sakamoto Wase" is controlled by different genetic systems.

The near-isogenic lines is being developed by a method of repetitive heterozygote selection method to determine the genetic basis of the insensitivity to ILD in the two land races in more detail. We tested the response of the ILD-sensitive families to FLD. As a result, all of the ILD-sensitive plants tested flowered normally under FLD. The result suggests that the two parents used may have *e3* for the insensitivity to FLD in common. Accordingly, "Miharu Daizu" and "Sakamoto Wase" appear to have different genotypes at *E4* locus. If so, there must be at least a gene which cancels the effect of *E4* or promotes the insensitivity to ILD under the presence of *E4*.

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Table 1. Frequency distribution of parents and F2 progeny on the date of the first appearance of an open flower (R1) under ILD in the cross between "Miharu Daizu" and "Sakamoto Wase".

	Date of the first appearance of an open flower (R1)											Total no.
	July			August							non-flowering	of plants
	16	20	24	28	1	5	9	13	17	21		tested
Miharu Daizu		2	8									10
Sakamoto Wase			10									10
F2	1	12	26	2	3	7	6	11	12	4	10	94

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Evolutionary Relationships between Changes in the Mitochondrial and Chloroplast Genomes in the Genus *Glycine* Subgenus *Soja*

Introduction

The cytoplasmic diversity in the genus *Glycine* subgenus *Soja* have been analyzed using restriction fragment length polymorphisms (RFLPs) with regard to chloroplast DNA (cpDNA; Shoemaker et al., 1986; Close et al., 1989) and mitochondrial DNA (mtDNA; Sisson et al., 1978; Grabau and Davis, 1992; Grabau et al., 1989, 1992; Hanlon and Grabau, 1995; Tozuka et al., 1998).

We have collected numerous wild soybeans (*G. soja*) growing in natural habitats and cultivated soybeans (*G. max*) in East Asia, and analyzed both mtDNA and cpDNA (Hirata et al., 1996; Tozuka et al., 1998), as well as the mechanisms that generate the RFLPs of mtDNA (Kanazawa et al., 1998). We have classified mitochondrial genomes of the genus *Glycine* subgenus *Soja* based on hybridization patterns in DNA gel-blot analyses using two mtDNA clones as probes (Tozuka et al., 1998). We employed three combinations of mtDNA probes and restriction endonucleases: *cox2* and *Bam*HI; *cox2* and *Hin*dIII; *atp6* and *Bam*HI, and classified the mitochondrial genomes of 1097 wild soybean plants into 18 types (Tozuka et al., 1998). In our classification, mitochondrial genome types were designated as combinations of types 'I' to 'VII', and types 'a' to 'k', based on hybridization patterns detected by *cox2* and *atp6* probes, respectively. Almost all the mitochondrial genome types detected in cultivated soybeans were also detected in wild soybeans. With regard to cpDNA, we have detected three types of cpDNA designated as types I, II and III, which are the same as those previously detected by Close et al. (1989), by using a cpDNA clone "H2", which contains a region close to inverted repeats within the large single copy region of cpDNA (Hirata et al., 1996; Abe et al., submitted), as a probe. In most cases, no variation in chloroplast genome was detected in the plants that belong to the same mitochondrial genome type: most of the mitochondrial genome type is coupled with only one of the three chloroplast genome types. Several exceptions, however, were detected. These were groups of plants that have the types mtIIIb, mtIVb or mtIVc, in which both types cpI and cpII were detected (for classification of genome types, see Table 1). In the present study, we report conserved structures of mitochondrial genome, which were revealed by using small interspersed sequence (Kanazawa et al., 1998) as a probe for DNA gel-blot analyses, within the plants that belong to these mitochondrial genome type. We also describe possible explanation for presence of common cpDNA variation in the plants that have different mtDNA configurations.

Materials and Methods

G. max and *G. soja* plants collected and maintained in our laboratory were used as materials. Extraction of DNA, and gel-blot analyses of DNA were done as described previously (Tozuka et al., 1998).

Results and Discussion

During the courses of our studies on RFLPs of mtDNA or cpDNA in the subgenus *Soja*, we found common variation in the chloroplast genome in plants that belong to different mitochondrial genome types, namely, types mtIIIb, mtIVb and mtIVc. We examined whether there are differences in mtDNA among the accessions of the subgenus *Soja* that belong to these mitochondrial genome types, in which two different chloroplast genome types, namely, cpI and cpII have been detected (see Table 2). We used a mtDNA fragment that contain a 299-bp repeated sequence (Kanazawa et al., 1998) as a probe for a DNA gel-blot analysis. The 299-bp repeated sequence was shown to be interspersed in 8 loci in the mitochondrial genome of 'B09002', an accession of *G. soja*, and to be used as sites of recombination event (Kanazawa et al., 1998), so that it allows us to detect variations at many loci by a single hybridization experiment. Total DNA extracted from each accession was digested with *Hin*dIII and hybridized with the probe. As a result, eight to ten hybridization signals were detected in each DNA (Table 2), suggesting that the sequence is present repeated in the mitochondrial genomes of respective plants. Signal intensity was highly varied among the signals found in each DNA, which reflects quantitative variations of various mtDNA molecules on which repeated sequences were located. Hybridization patterns differed each other among DNAs of different genome types, while no conspicuous difference was detected in the plants that belong to the same mitochondrial genome type (Table 2). This result may indicate that the genome is extensively conserved among accessions that have the same mitochondrial genome type (i.e., the same configurations around *cox2* and *atp6*), although both cpI and cpII types were found in each mitochondrial genome type. A possibility that all the repeated sequences were present at the 5' regions of *cox2* or *atp6* is not likely because some hybridization signals detected by using the 299-bp repeated sequence as a probe were not hybridized neither with the *cox2* probe nor with the *atp6* probe. In the case of 'B09002', five of eight loci of the repeated sequence actually corresponded to the 5' region of *cox2* or *atp6*, while the other three loci did not (Kanazawa et al., 1998).

Presence of both the cpI and cpII types in the groups of plants that have the mtIIIb, mtIVb and mtIVc types suggests that the same change(s) in either the chloroplast genome or the mitochondrial genome occurred repeatedly during evolution. Recently, we found that differences in mtDNA at the *cox2* or *atp6* loci between different accessions can be explained by recombination events across the 299-bp or a 23-bp repeated sequences (Kanazawa et al., 1998). The recombination events may be followed by selective amplification (or loss) of subgenomic recombinant mtDNA molecules, on which different configurations of mtDNA around *cox2* or *atp6* are located. This may explain simple gain or loss of certain restriction fragments of mtDNA that appeared in a DNA gel-blot analysis. It does not seem difficult to postulate that these changes can be occurred repeatedly during evolution. On the other hand, the RFLPs that distinguish types cpI-III were found to be due to point mutations (unpublished data). In general, point mutation may occur

randomly, and therefore, it is not likely that the same mutation at the same site has been occurred repeatedly during evolution.

With regard to the three mitochondrial genome types that were found to be coupled with both the type cpII and type cpI, the types mtIIIb and mtIVc were very rarely detected, while the type mtIVb was detected very frequently in natural population in East Asia. If we assume that the variation in the mitochondrial genome occur repeatedly, the process of evolutionary changes in the genomes can be explained as follows: first, a change from type cpII to type cpI, which was implied by Close et al. (1989), was occurred in plants that carry the predominant type, mtIVb; and second, types mtIIIb and mtIVc were generated from both type cpII-mtIVb and type cpI-mtIVb. Interestingly, the types mtIIIb, mtIVb and mtIVc were located very closely in the phylogenetic trees of mitochondrial genome types that were constructed based on hybridization profiles of mtDNA (Kanazawa et al., submitted). This may indicate that changes in structure of mtDNA among these three types involve small number of steps. We have found several predominant types of mtDNA other than the type mtIVb in East Asia. Although these mtDNA types were not detected with more than one type of cpDNA, at present, we cannot completely deny the possibility that a change from type cpII to type cpI occurred in plants having mitochondrial genome type other than type mtIVb. We have also found another mtDNA type that couples with more than one type of chloroplast genome, such as type mtVb, which distributed mainly in Korea and was found with either types cpIII or cpII. Our ongoing project of cpDNA sequencing may reveal whether types cpI and cpII have occurred in plants having types mtIVb and mtVb, respectively.

The high level of recombination activity in mitochondria, as well as quantitative variations of mtDNA, which may include shifts in equilibrium between different subgenomic mtDNA molecules, have resulted in highly complex organization of the genome in higher plants. Although some of the mechanisms that generate mtDNA variations in the subgenus *Soja* have been analyzed (Chanut et al., 1993; Moeykens et al., 1995; Kanazawa et al., 1998), a large part of the evolutionary changes of the genome remains unknown. However, apparent geographic clines of the distribution of mitochondrial genome types (Tozuka et al., 1998) suggest that analysis of the changes in the composition of mtDNA provides useful tool to study the evolution of the subgenus *Soja*.

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Table 1. Hybridization signals characteristic of the chloroplast and mitochondrial genome types (size: kb)

Genome type	Probe	H2	
	Enzyme	*Eco*RI	*Cla*I
cpI		4.8	2.4, 1.1
cpII		4.8	3.5
cpIII		2.5	3.5

Genome type	Probe	*cox2*		*atp6*
	Enzyme	*Hind*III	*Bam*HI	*Bam*HI
mtIIIb		1.2	8.5	5.0, 2.9
mtIVb		3.5	8.1	5.0, 2.9
mtIVc		3.5	8.1	5.0
mtVb		5.8	8.1	5.0, 2.9

Table 2. List of accessions and hybridization profiles probed with the 299-bp repeated sequence

Accession	mtDNA type	cpDNA type	Species	Origin	Hybridization profile (size: kb)
B08007	IIIb	I	*G. soja*	Shimane, Japan	8.5, 5.5, 4.9, 3.9, 2.8, 2.3, 1.6, 1.1, 1.0
B08008	IIIb	I	*G. soja*	Shimane, Japan	8.5, 5.5, 4.9, 3.9, 2.8, 2.3, 1.6, 1.1, 1.0
Amakusa	IIIb	I	*G. max*	Kumamoto, Japan	8.5, 5.5, 4.9, 3.9, 2.8, 2.3, 1.6, 1.1, 1.0
B07001	IIIb	II	*G. soja*	Kagawa, Japan	8.5, 5.5, 4.9, 3.9, 2.8, 2.3, 1.6, 1.1, 1.0
B09015	IVb	I	*G. soja*	Kumamoto, Japan	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Chashouryuu	IVb	I	*G. max*	Hokkaido, Japan	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Harosoy	IVb	I	*G. max*	Canada	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
B07108	IVb	II	*G. soja*	Kochi, Japan	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
B02117	IVb	II	*G. soja*	Iwate, Japan	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
B00058	IVb	II	*G. soja*	Chungchongbuk-do, Rep. Korea	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Akasomedaizu	IVb	II	*G. max*	Korea	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Kurodaizushouryuu	IVb	II	*G. max*	Korea	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Hulanliuyueqi	IVb	II	*G. max*	Heilongjiang, China	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Kinaikurosengoku	IVb	II	*G. max*	Nara, Japan	8.5, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.0
Wasenatsu	IVc	I	*G. max*	Saga, Japan	8.5, 6.8, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.1, 1.0
Sakamotowase	IVc	I	*G. max*	Hokkaido, Japan	8.5, 6.8, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.1, 1.0
B00101	IVc	II	*G. soja*	Chungchongbuk-do, Rep. Korea	8.5, 6.8, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.1, 1.0
Takayamazairai	IVc	II	*G. max*	Gifu, Japan	8.5, 6.8, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.1, 1.0
Hunanniumaohuang	IVc	II	*G. max*	Hunan, China	8.5, 6.8, 5.2, 3.9, 3.5, 2.8, 2.3, 1.6, 1.1, 1.0

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Spontaneous Mutation for Late-Flowering and Its Effect on the Reproductive Organs in Japanese Soybean Germplasm

Introduction

Maturity and number of flower bud per node are very important factors in soybean yields and range of its adaptability.

A late-flowering soybean plant (MF plant) derived from cross with early-flowering and few flower bud parents was observed in $F_{7:8}$ rows at the Tokachi Agricultural Experiment Station (Tokachi AES), Memuro, Japan in 1993. MF plant has later flowering date, long peduncle in florescence type, and many flowers per node than each of parents. We first thought that it still showed heterosis such as we often observed in early generation. However, with the uniformity of other phenotypes in advanced lines of F_9 to F_{10}, we assumed the MF lines might be a spontaneous mutation for late-flowering.

The objectives of this study were to (1) determine the inheritance of late-flowering; (2) investigate the effect of late-flowering on the inflorescence type, length of peduncle, number of flower buds in MF lines.

Materials and Methods

The true-breeding and segregating MF lines derived from $F_{8:11}$ and $F_{8:12}$ lines of the cross TC6225 (Tokei727 x Toiku214) were used in this study. Tokei727 and Toiku214 were soybean breeding lines in Tokachi AES. Tokei724 was similar to Toiku214 in maturity and growth habit. Near-isogenic lines for maturity, Harosoy, L64-4103, and Clark and L67-1474 were obtained from Dr. R. L. Nelson, USDA-ARS (Urbana, Illinois) as compared with MF plants.

MF lines were classified into nonsegregating-early, segregating, or nonsegregating-late according to the days to first flower after planting in summer of 1996 at Tokachi AES, Memuro in Hokkaido, Japan and 1997 at the Bruner Farm near Ames in Iowa, USA. The data of phenotypic traits, peduncle length, and flower bud number in the middle portions of the plant were collected in summer of 1997 at the Bruner Farm. The chi-square test was used to measure goodness of fit to expected genetic ratios for flowering.

Results and Discussion

F_{11} families derived from segregating MF6, MF7, and MF8 lines gave a good fit to the expected 1:2:1 ratio of nonsegregating-late: segregating: nonsegregating-early in flowering. The chi-square tests of segregation ratios of MF lines and progenies showed a good fit to the expected ratio for a single gene pair with lateness being dominant to earliness (Tables 1 and 2). F_{12} family of MF7-13 derived from segregating MF7 lines also gave a good fit to 1:2:1 ratio, confirming that the early-flowering MF7-1301 and late-flowering MF13-1357 lines are near-isogenic lines with single gene for lateness of flowering (Tables 3 and 4). The days to first flower of MF7-1357 and MF7-1321L (segregating to late-flowering) were quite similar to that of Clark ($e_1E_2E_3E_4e_5$ *T* Dt_1). The days to first flower of MF7-1301, MF7-1321E (segregating to early-flowering), Tokei727, and Toiku214 were 3-4 days earlier than Harosoy ($e_1e_2E_3E_4e_5$ *t* Dt_1). In comparison with near-isogenic lines of Harosoy and Clark for the maturity, the unknown maturity alleles of MF lines seem to be similar to the maturity alleles at E_2 locus (Bernard, 1971). However, further studies are necessary to identify a locus of the MF maturity gene (Table 5).

The length of peduncle and number of flower buds of MF lines differed in flowering. The near-isogenic lines of Harosoy and Clark also showed the same results of MF lines (Table 5). Thus, the elongation of peduncle inflorescence and large number of flower buds per node might be due to the effect of late-flowering and maturity allele. VanSchaik and Probst (1958) reported that an inflorescence type of soybean was linked with maturity and height, and the long peduncle inflorescence and large number of flower buds per node were associated strongly with late maturity. Therefore, we are conducting further studies to confirm the genetic relationship between the flowering and inflorescence type loci in MF lines.

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Table 1. Segregation for days to flowering in F_{11} lines of the MF7 family (Memuro 1996)

Line	N[1]	Days to first flowering[2]												No. of plants		χ^2	P
		60	63	66	69	72	75	78	81	84	87	90	93	Early[3]	Late[4]	(1:3)	(df=1)
MF7-23	33	33												33	0	-	
MF7-13	88	15	8					1	12	46	6			23	65	0.06	0.81
MF7-05	217									103	108	6		0	217	-	

1) N = number of plants observed.
2) Days to first flower after planting date of May 20.
3) Early includes the flowering days from 60 to 73 after planting.
4) Late includes the flowering days from 74 to 94 after planting.

Table 2. Chi-square tests for flowering date in F_{11} lines of the segregating MF families (Memuro 1996)

Family	No. of lines					No. of plants in segregating lines			
	Non-seg early	Segregating	Non-seg late	χ^2 (1:2:1)	P (df=2)	Early	Late	χ^2 (1:3)	P (df=1)
MF6	6	11	11	3.07	0.22	187	521	0.75	0.39
MF7	6	13	8	0.46	0.79	187	531	0.42	0.52
MF8	11	9	6	4.38	0.12	142	529	5.27	0.02

Table 3. Segregation for days to flowering in F_{12} plants of MF7-13 lines (Ames 1997)

Line	N[1]	Days to first flowering[1]											No. of plants		χ^2 (1:3)	P (df=1)
		56	58	60	62	64	66	68	70	72	74	76	Early[3]	Late[4]		
MF7-1301	46	46											46	0	-	*
MF7-1321	41	5	7	1			3	3	8	4	5	5	13	28	0.98	0.32
MF7-1357	37						2	4	1	23	7		0	37	-	*

1) Number of plants observed.
2) Days to first flower after planting date of May 10.
3) Early includes the flowering days from 56 to 60 after planting.
4) Late includes the flowering days from 66 to 76 after planting.

Table 4. Chi-square tests for flowering date in F_{12} lines of segregating MF7-13 family (Ames 1997)

Family	No. of lines					No. of plants in segregating lines			
	Non-seg early	Segregating	Non-seg late	χ^2 (1:2:1)	P (df=2)	Early	Late	χ^2 (1:3)	P (df=1)
MF7-13	16	35	15	0.27	0.87	363	1023	1.05	0.31

Table 5. Phenotypic traits of MF near-isogenic lines (Ames 1997)

Strains	Genotype	N[1]	Days to flower[2]	DFRH[3]	Inflorescence[4]		No. of flower buds[4] per node
					Type[5]	Length (mm)	
MF7-1301	Unknown *t dt1*	10	55 ± 0.2	-4	P	17.4 ± 2.4	7.2 ± 1.0
MF7-1357	Unknown *t dt1*	37	71 ± 0.7	+11	P	39.5 ± 6.7	10.0 ± 1.4
MF7-1321E	Unknown *t dt1*	13	55 ± 0.6	-4	P	14.5 ± 8.6	5.4 ± 1.4
MF7-1321L	Unknown *t dt1*	28	69 ± 1.1	+10	P	35.0 ± 6.8	9.3 ± 1.1
P1 Tokei727	Unknown *t dt1*	10	56 ± 0.2	-3	P	7.5 ± 2.9	6.9 ± 0.8
P2 Toiku214	Unknown *t dt1*	10	55 ± 0.3	-4	P	14.8 ± 3.9	7.7 ± 1.1
Harosoy	*e1e2E3E4e5 t Dt1*	10	59 ± 0.3	0	S	——	7.1 ± 1.4
L64-4103	*E1e2E3E4e5 t Dt1*	10	80 ± 0.3	+21	P	53.4 ± 11.9	11.7 ± 2.4
Clark	*e1E2E3E4e5 T Dt1*	10	69 ± 0.2	+10	P	4.7 ± 4.7	6.9 ± 1.0
L67-1474	*E1E2E3 E4e5 T Dt1*	10	89 ± 0.3	+31	P	39.2 ± 3.9	16.6 ± 1.8

1) Number of plants observed.
2) Days to first flower after planting date of May 10.
3) DFRH; Days to flower relative to Harosoy
4) Peduncle length and number of flower buds were observed in the middle portions of the plant.
5) Inflorescence type was classified as pedunculate (P) or sub-sessile (S).

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Genetic Improvement of Soybean Through Induced Mutations

Pakistan is facing acute shortage of edible oils, with domestic production meeting only 20% of the total requirements. This deficiency is increasing by 13-15% every year. To improve this grave situation soybean has bright prospects of emerging as the most promising oil crop for Pakistan to achieve self sufficiency in edible oils. Realizing the importance of soybean and vital role of mutation breeding in the development and release of new improved cultivars (Micke 1985), mutation breeding work was initiated on soybean at AEARC, Tando Jam with the following objectives.

Objectives

i) Breeding for earliness in flowering and maturity
ii) Uniform maturity.
iii) Resistance to seed shattering
iv) Disease resistance (Rust resistance)
v) Wide adaptability
vi) High yield potential

Materials and Methods

Pure and air dried seeds of three soybean varieties, including two early group varieties (Steeli 5/1 and Columbus) and one late maturing group (Improved Pelican) were irradiated with different doses of Y-rays ranging from 10-40 kR. M1 generations was raised during 1984 and M2 generation in 1985. Desirable selections were made from M2 population on the basis of phenotypic performance and recorded morphological observations in the field and laboratory. These putative mutants were confirmed for their breeding behaviour. True breeding lines were evaluated in preliminary trials, micro yield trials and advanced line trials. Here the results of these mutants lines in the Station Yield Trials are presented.

Summary of previous work

Quite a few mutants having earliness and uniform maturity along with high yield potential have been developed (Rajput and Siddiqui 1983, Rajput and Siddiqui 1986, Rajput et al 1987, Rajput and Sarwar 1988, Rajput and Sarwar 1989). Some of them are being evaluated at different advance stages of testing. In this report latest performance of mutants developed from three leading varieties is being presented and discussed.

i) Mutants developed from variety steeli 5/1

Seven mutants along with parent variety Steeli 5/1 were evaluated. Morphological as well as grain yield data (Table 1) indicate that mutant 20/139 (2065 kg/ha) produced significantly higher grain yield and ranked first followed by mutant 30/31 (1951 kg/ha) and mutant 20/105 (1928 kg/ha), whereas parent variety (Steeli 5/1) produced 1493 kg/ha grain yield and ranked seven in the trails.

ii) Mutants developed from variety improved pelican

Mutants having determinate growth habit, synchronous maturity, dwarf cum compact-plant type and high yield potential were derived from variety Improved Pelican having tall, indeterminate plant growth habit and non synchronous pod maturity.

Eight genotypes (7 mutants + parent) were studied in this trial. As per results (Table 2) mutant IP. 4/85 gave significantly higher grain yield (2491 kg/ha) followed by IP 20/I/85 (2318 kg/ha). IP 6/85 (2309 kg/ha), IP 12/85 (2031 kg/ha) and IP 2/85 (1988 kg/ha). The mother variety Improved Pelican produced 1466 kg/ha grain yield.

iii) Mutants developed from variety columbus

Five mutant lines along with parent variety Columbus were evaluated in this trial (Table 3). Mutant C 5/1 gave highest grain yield (2188 kg/ha) followed by C 18 (2163 kg/ha) and C 1 (2031 kg/ha). The mother variety Columbus produced 1926 kg/ha grain yield and remained at bottom in performance. However, the yield differences were not significant.

Conclusions

True breeding lines developed from the present project have exhibited impressive yield potential in preliminary field evaluation. Some of these lines have quite high yield potential. However, further studies are needed to evaluate them under different ecological zones of Sindh, Pakistan. It is expected that at least some of them would be released \either directly as new varieties or incorporated into conventional breeding programmes. Induced mutations have also generated new germplasm for useful agronomic traits.

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Table 1. Performance of soybean mutants derived from variety Steeli 5/1 spring.

Genotype	Days to flower	Days to mature	Plant height (cm)	100 grain weight (g)	Grain yield (kg/ha)	Rank
Mutant 30/IL	33	91	57	14.90	1510	6
Mutant 30/31	33	91	47	14.06	1951	2
Mutant 20/105	32	92	47	14.13	1928	3
Mutant 20/139	33	92	49	15.03	2065	1
Mutant 40/1	33	91	55	15.05	1805	5
Mutant 30/1	32	90	43	13.66	1805	5
Mutant 20/32	33	90	48	14.10	1893	4
Steeli 5/1 (Parent)	33	90	42	14.29	1493	7
LSD 5%					373.9670	

Table 2. Performance of soybean mutants evolved from Improved Pelican (Kharif).

Genotype	Days to flower	Days to mature	Plant height (cm)	100 grain weight (g)	Grain yield (kg/ha)	Rank
IP 20/85	67	131	96.30	13.17	1328 E	8
IP 4/85	69	129	92.2	15.16	2491 A	1
IP 20/1/85	68	130	92.55	12.37	2318 AB	2
IP 2/85	68	128	54.77	15.12	1988 BC	5
IP 2/1/85	69	128	92.95	15.52	1718 CD	6
IP 12/85	69	130	102.85	12.71	2031 BC	4
IP 6/85	68	129	92.30	12.54	2309 AB	3
IP (Parent)	68	131	125.70	14.53	1466 DE	7

Table 3. Performance of soybean mutants evolved from variety Columbus (Spring).

Genotype	Days to flower	Days to mature	Plant height (cm)	100 grain weight (g)	Grain yield (kg/ha)	Rank
Mutant C 1	31	97	39	14.80	2031	3
Mutant C 3/1	32	96	46	13.44	1996	4
Mutant C 5/1	32	95	54	14.78	2188	1
Mutant C 16	33	97	44	13.29	1979	5
Mutant C 18	33	94	49	13.40	2153	2
Columbus (Parent)	33	94	41	13.70	1926	6

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Changes in the Soybean Cotyledon Storage Proteins Induced by the Growth Regulators during the Dedifferentiation

Introduction

The structure and concentrations of growth regulators (GR) play a great role in the *in vitro* culture. A rate of incorporation of the synthetic GR in the plant cells metabolic pathway increases when their structure is the near to the native one. The content of the native GR of the auxine type (IAA) in the plant cells depends on the L-tryptophane level, which is converted in the indolylpyruvate pathway on the IAA (Sebánek et al. 1991). Free cytokinins and their precursors identified in the plant tissue are usually contained in a form of isopentenyladenine, dihydrozeatine, zeatinriboside and various topoline isoforms (Strnad et al. 1994). Protein and morphological analyses of the transgenic tobacco and soybean plants expressing the morphoregulatory genes *ipt, iaam and* iaah, causing to the overproduction of cytokinin and auxin, showed that the level of their gene products was regulated with the gene families (Beinsberger et al. 1991; Romano et al. 1993; Hlinková et Ondrej 1994; Hlinková 1994). Conversion of differentiated cells from various plant tissues to dedifferentiated one is affected by the concentration gradient between the endogenous and exogenous GR, influencing the gene expression and the entire protein spectrum. GR of the benzothiasolinone type have biological activity (Sekerka et al. 1989). One from them is the auxinoid 3-benzyloxycarbonylmethyl-2-benzothiasolinone (Mr=299,35; BB). This organic compound is an active component of the commercial preparation Rastim-30 DKV. The optimal concentration (1μM of BB) was used by callus cultivation of the various plant species including soybean (Hlinková 1990; 1993). This concentration did not affect a duration of the mitotic cycle and a mitotic activity by legumes (Hlinková et Belková 1991) and had no cytogenic and cytotoxic effects (Miadoková et al. 1995). The aim of our work was to find changes in the gene expression and in the storage proteins induced by various auxinoids during the cell dedifferentiation of soybean explants.

Materials and Methods

Soybean seeds of cv. Maple arrow line H-12 (obtained from Dr. Volenberg, Institute of Plant Breeding Ottawa, Canada) and cv. Aida (Plant Breeding Station Horni Mostenice, Czech Rep.) were surface sterilised for 3 min. in 70% et-OH and washed 3-times with the sterile double distilled water. After 24h imbibition in the sterile water they were repeatedly sterilised for 15 min in 5% chloramine B. Three times washed with the sterile double distilled water aseptical seeds were placed on the medium MS without growth regulators (Murashige et Skoog 1963). Cultivation took place in the growth chambers at 23±1 °C with illumination 2 200 lx during 16h. Six-week-old aseptically cultivated soybean plants were completely cut into the 3-4 mm transversal slices and placed on the experimental media mER containing 50 nM of kinetin and 3-benzyloxycarbonylmethyl-2-benzothiasolinone in the concentration 1mM, 1μM and 1nM (Hlinková 1993). The control medium contained 5μM 2,4-D and the same concentration of kinetin. The gene expression was observed in the primary callus induced on the slices from the middle part of cotyledons of both soybean cultivars after 28 days cultivation under the identical conditions.

General soluble proteins were extracted in the non-denaturating 0.1M Naphosphate buffer pH7 (Hlinková et al. 1995). The quantitative content of the soluble proteins in the samples were detected by Bradford's spectrocolorimetric method (Bradford 1976). Anodic non-denaturated proteins were separated on the 12.5% discontinual polyacrylamide gel system according to Smith (1988). Cathodic proteins were separated on the 15% discontinual polyacrylamide gel system according to Reisfeld (1962). Denaturated proteins for SDS-PAGE were prepared according to Laemmli (1970) and analysed on the 13% discontinual SDS-PAGE gel. Anodic and cathodic proteins with the peroxidase activity (E.C. 1.11.1.7.) were visualised by Vallejos' method (Vallejos 1983). Gels were stained with AgNO3 according to method of Nesterenko et al. (1994). Wide molecular mass protein standard from Bio-Labs (New England, U.S.A.) was used as a marker of Mr for SDS-PAGE. Haemoglobine (H: Mr~68kda; polypeptides: 52; 30; 14.5 and 14.0 kDa, Serva Heidelberg Germany), bovine serum albumine (Al: Mr~66.7 and 66.2 kDa; Imuna Sarisske Michalany, Slovakia) and lysozyme (Ly: Mr~14.3 kDa; Sigma, St. Louis,U.S.A.) were used as molecular mass standards for A-PAGE. Cellulase Onozuka R-10 (Mr~76 and 42 kDa; Serva Heidelberg) and native soybean trypsin inhibitor (21 kDa) were used as molecular mass standards for C-PAGE. Electrophoresis was done in the Midi Protean gel apparatus of LKB Pharmacia (Uppsala, Sweden). The experiments were repeated 3-times.

Results and Discussion

For a long time a soybean has been used as a model object of *in vitro* cultures (Gamborg et al. 1981; Lazierri et al. 1990; Hlinková 1993). Various problems, such as cytogenetic stability/instability in the *in vitro* cultures, physiological and morphogenic changes induced by stress and mutagenic factors, possibility of embryogenesis and organogenesis induction, were studied. Changes in the gene expression induced by the various GR during calliogenesis were not investigated. We concentrated on the morphological and molecular changes by the dedifferentiation of soybean explants. The differences of used soybean genotypes (2n=40 and content of DNA per somatic cell 2.35ng Dolezal et Hlinková, not published) were found by the mass of their seeds, content of soluble storage proteins, fertility, germination, height of the plants, leaf surface per plant and quantitative content of chlorophyll *a/b*. Some of these genetically

controlled characteristics manifested themselves during the dedifferentiation under *in vitro* conditions. Topography of soybean segments showed that the cell conversion to the cell with higher proliferation activity was on the most explants from the young soybean plants cultivated on the medium with 5μM of 2,4-D (ratio of exogenous GR=100,Hlinková 1993). Practically all explant cells were converted to the primary callus whose volume was 3-5 times larger than the volume of the initial segments was. Explants derived from cv.A gave the callogenesis predominantly on the cut surfaces of the explants. Genetic differences of used soybean genotypes were made evident by the rate of cell conversion of segments from specialised tissues. The volume of the primary callus on the corresponding cotyledon segments for cv. A decreased about 50% as compared to cv.MA. Concentration of 1mM BB in the medium (ratio of exogenous GR=2.10^4) made input in the cell division slower (Hlinková et Belková 1991). The primary callus volume was smaller and the cells changed their colour from white-yellow to orange, probably as the results of different secondary metabolites contained in the vacuoles. The type of the dedifferentiated tissues was not changed by both cultivars. Explants, predominantly from the cotyledon middle part, contained cells in that stage of the mitotic cycle where the gradient of the exogenous and native endogenous GR controlling the check point of G1/S and G2/M of mitotic cycle can change both the rate and the direction of their proliferation. Concentrations of 1 μM BB (ratio of the exogenous GR=2.10^{-2}) and 1nM BB (ratio of exogenous GR=2.10^{-2}) in the cultivation medium did not induce callogenesis. The content of chlorophyll a in the explants from cotyledons in both cultivars increased as compared to the adequate segments growing on the medium with the 2,4-D as well as to segments from the plants growing on the medium MS without growth regulators. These results showed that the content of chlorophyll a in explants depended on the concentration of the exogenous cytokinin and their ratio to auxin in the cultivation media. The level of the endogenous cytokinin controlled the content of chlorophyll a in the leaves of the transgenic plants carrying *ipt* gene (Hlinková et Ondrej 1994, Hlinková et al. in press). Segments from the region of the root neck induced rizogenesis by the both genotypes. The smallest concentration of BB induced the organogenesis of adventitious shoots from the basal cells of cotyledons and the elongation growth of the apex segments. The high exogenous cytokinin level in the cultivation medium was responsible for these morphogenic processes.

Depending on their concentration in the medium and the dedifferentiation effects, growth regulators influenced the level of the soluble proteins (SP) in the middle segments of the cotyledons. The concentration of SP in these segments depended on both the rate of the cell input into the cell dedifferentiation and storage protein utilisation in the comparison with the identical segments from the plants cultivated *in vitro* conditions on the medium MS without GR. The highest level of the SP was measured in the segments of both cultivars cultivated on the medium mER with 1 nM BB (Table 1).

The qualitative analyses of the protein patterns showed that the highest amount of SP had the storage proteins from the classes of lectines and glycines. Maple arrow line H-12 differed in the addition of the level of the soybean trypsin inhibitor. The concentration of the SP in the primary callus was 50% lower as compared to the middle part of the cotyledon segments cultivated on the medium with 1 nM BB. 1 mM BB did not influence the level of the SP preferably in cv. Aida. The genetic background in the SP was reflected by both auxinoides used. The qualitative and quantitative content of the SP in the middle cotyledon segments as well as in their residue tissue changed *in* vitro conditions according to the genotype, concentration of the GR in the medium and the chemical structure of the auxinoids used. Differences in the protein patterns compared to the middle cotyledon segments from the soybean plants of the same age and cultivated *in vitro* conditions without GR are showed for anodic and denaturated proteins in the Tables 2 and 3.

Genotype differences in the primary calli were registered by α, β, γ subunites of conglycine (Mr~67- 9OkDa). Strong quantitative changes were investigated in the content of α. and β trypsin inhibitor subunits (Mr~20.2 and 20.04 kDa), acid phosphatase (Mr~53kDa), β-amylase (Mr~57 kDa), lectine (Mr~110 kDa; polypeptides~54 kDa) and in proteins connected with phosphorylation and dephosphorylation reactions and cdc-complexes (p34 and p36). Differences were registered by chlorophyll *a/b* binding proteins (p52; p44; p3O, p26 and p17). The influence of various BB concentrations was observed by p5O protein. Specific protein (p24) was observed in the residue part of cotyledons by the dedifferentiation on the medium with 2,4-D.This protein probably belongs to the class of auxin-binding proteins. Protein with identical molecular mass and auxin-binding function was identified by Tian et al. (1995) in the intracellular proteins of Zea mays L. coleoptile. Jones and Herman (1993) located it at the plasma membrane. The p93 protein was classified as a cytokinin binding protein (Sebánek et al. 1991). This protein was found only in the protein pattern for MA segment growing on the medium with 1 μM BB. The differences between α and β subunits of soluble vegetable proteins (p32 and 35) registered by Susman (1994) in the young leaves and soybean cotyledons were not detected. .

Various chemical structures and concentrations of the exogenous auxinoids influenced peroxidase isozyme patterns in the both cathodic (B) and anodic (A) pH regions. Their quantitative content in various types of tissue is given in the Table 4.

Isozyme patterns showed that auxinoids increased the protein content with peroxidase activity mainly in the primary callus which is connected with the gene expression by the cell dedifferentiation, while in the cotyledons of the plants cultivated *in vivo* there were no proteins with peroxidase activity detected. The genotype differences were observed in the cathodic proteins too. BB induced synthesis of new proteins with peroxidase activity. Their molecular mass was 60, 90 and 100 kDa. Changes in the peroxidase isozyme patterns indicated that

catabolic reactions of the both auxinoids in the plant metabolism are going with various enzymatic pathways. Receptor channel is probably the same for both auxinoids. During the input of GR into the intracellular space the great importance have P-proteins (Mr~180 kDa) find on the outside surface of the cytoplasmatic membrane, ATP-ses, peroxidases and superoxiddismuses. These proteins with high probability at the first step degraded used complexity organic compound.

On the basis obtained results we can note that;

I - gene expression in the cotyledons of the studied soybean genotypes is due to the speed of utilisation of storage proteins depending on the epigenetic factors;

2 - gene expression by the dedifferentiation depends on the chemical structure of the used auxinoids and their concentration in the cultivation medium;

3 - gene expression in the primary calli reflected genotype differences, pleiotropic effects of used auxinoids as well as their extra and intracellular gradients;

4 - quantitative content of storage protein in the middle part of cotyledon segments of both genotypes changed in the dependence of the dedifferentiation level;

5 - BB compared to 2,4-D produced greater effect on the amount of the protein p5O and soybean trypsin inhibitor subunits in the primary soybean calli;

6 - quantitative differences between the proteins p44, p34, p25, p22 and pl6 indicated the connection of the used growth regulators with the phosphorylation and dephosphorylation reactions and cell transition in the mitotic cycle via check-point G1/S and G2/M of the cell division.

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Table 1. Quantitative content of the soluble proteins in the extracts from various soybean tissues

cv	S	0		5 μM 2,4-D		1 mM BB		1 μM BB		1 nM BB	
		C_n	C_{iv}	C_r	p.c.	C_r	p.c.	C	p.c.	C	p.c.
MA	66	0.7	1.62	0.94	1.6	-	1.8	1.94	-	3	-
[mg/gl	±1.5	±0.0	±0.2	±0.0	±0.0		±0.0	±0.0		±0.7	
		5	4	7	8		5	9			
A	48	0.6	0.87	0.74	0.61	0.7	1.6	1.53	-	1.9	
[mg/gl	±0.9	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0		±0.5	
		7	5	3	5	2	1	3			

cv.- soybean cultivar;
S-seed;
n-cultivation *in vivo* conditions,
i.v.-Cultivation in vitro condition;
C-cotyledon;
p.c.-primary callus;
r-residue cotyledon tissue part;
protein concentration (mg) was calculated on the unit of the fresh mass tissue (g).

Table 2. New anodic proteins identified in the middle part of soybean cotyledon after dedifferentiation

Tissue	Aida	Maple arrow H-12
C $(2,4\text{-D})_r$	220;120;100;69;45;34;30;28;24;14;	41.5;36;34;32;30;27;26;15.5;12.5;
p.c. (2,4-D)	120;110;69;28;27;17;15;12;	110;67;36;34;32;21;18;17;
C (1mM BB)	120;110;90;31.5;25;23;17;14,	——
p.c. (1mM BB)	120;110;90;43-41;35;25-24;17;15;	110;90;72;43-42;36-34;42,30,17;
C (1μM BB)	120;110;69;43.5;25;23.5;21;17;15-14	77;73;60;45;28;25;24;17;15.5
C (1nM BB)	120; 100;69;53;43.5;25;23.5; 17;14.5-14	88;78;75;68;60;55;53;47;40-41;34;28-24;

C-cotyledon;
p.c.-primary callus;
r-residue tissue part of cotyledon

Table 3. New polypeptides identified in the SDS-PAGE electrophoretograms of the denaturated soluble proteins

Tissue	Aida	Maple arrow H-12
C $(2,4\text{-D})_r$	43;41-138-120.2;lb.b	37;31;30;28;26.5-125.5;24-23;20.2;15.5
p.c. (2,4-D)	50;41;26-25;17;	126;77;60;50;35.5;30;28;23;20.2
C $(1\text{mM BB})_r$	69;67;60;43-42;37;28;24;20.2;16;	——
p.c. (1mM BB)	69;60;50;43;42;34; 17;	77;60;50;41;36-35;28;17;
C (1μM BB)	43-42;38-37;20.2;15;	93;39-36;28;17-16;
C (1nM BB)	66-65;60;53;50;37;27;19;17;16;	86;80;53;50;45;38;36;20.2;17

designation symbols as above

Table 4. The peroxidase content in the soybean tissue culture in the different phases of dedifferentiation

cv.		0		5 μM 2,4-D		1 mM BB		1 μM BB		1 mM BB	
	PRX	C_n	C_{iv}	C_r	p.c.	C_r	p.c.	C	p.c.	C	p.c.
MA	A	-	4	4	10	-	12	8	-	5	-
	B	-	2	3	4	-	4	5	-	5	-
A	A	-	4	4	6	4	6	5	-	3	-
	B	8	8	8	3	8	5	6	-	2	-

PRX–peroxidases (E.C. 1. 1 1. 1. 7);
C-cotyledon;
p.c.-primary callus;
A-anodic peroxidase isozymes;
B-cathodic peroxidase isozymes;
n-cultivation *in vivo* conditions;
iv - cultivation *in vitro* condition;
cv.- cultivar;
r- residue part of middle segment of cotyledon

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Variations Between and Within Maturity Groups of Soybean Genotypes for Biomass, Seed Yield, and Harvest Index

Abstract

Eighty two soybean [Glycine max (L.) Merr.] genotypes from maturity groups (MG) III to VIII were evaluated during 1992 & 1993 for determining the inter- and intra-maturity group variations for biomass, seed yield, and harvest index (HI) (the ratio of seed yield to the above ground total biomass); and identify genotypes superior in biological efficiency. In both years, significant variations were found within and between MGs for the parameters measured. The mean biomass ranged from 5.9 for MG III to 12.8 Mg ha^{-1} for MG VIII in 1992 and from 4.6 for MG III to 6.9 Mg ha^{-1} for MG VIII in 1993. In this study, the two-year mean biomass yields showed a consistent increase from MG III through VIII, that is, the higher the MG, the higher the biomass. The mean plant biomass for soybeans in MG VIII was 76.7% more than that for MG III. In 1992, 'Lee' produced the highest yield (6.3 Mg ha^{-1}). In 1993, 'Tanbagura' was the highest yielding (3.3 Mg ha^{-1}) genotype. The two-year mean yield for Miles (MG III) was 4.6 Mg ha^{-1}. The mean seed yield ranged from 2.7 (MG III) to 4.1 Mg ha^{-1} (MG VI) during 1992 and from 1.6 (MG IV & V) to 2.5 Mg ha^{-1} (MG VIII) during 1993. The mean HI ranged from 17 (MG VIII) to 64% (MG IV) in 1992 and from 24 (MG VI) to 51% (MG III) in 1993. During both years, seed yield was significantly correlated with biomass (r= 0.61*** to 0.87***) and HI (r=0.37** to 0.68***). The HIs of soybean genotypes within MGs VII and VIII were lower than that of other MGs, but these genotypes produced higher seed yields because of higher biomass compared to MGs III through VI. The study showed that soybean seed yields can be enhanced by breeding for increased biomass and HI.

Introduction

Identifying plant traits that contribute to crop productivity is essential for developing high yielding cultivars (Salman and Brinkman, 1992). Past yield enhancements in wheat (Triticum aestivum L.) have been attributed to increase in HI (Gifford et al., 1984). Gay et al., (1980) reported that partitioning of photosynthates to economic sinks contributed to yield differences among soybean genotypes. Soybean yield was reported to be correlated with HI (Bhardwaj and Bhagsari, 1989). But Salado-Navarro et al., (1993) found no correlation between yield and HI of soybean.

The data on biological yield, HI, and grain yield may be collected for an analysis of yield (Donald and Hamblin, 1976; McVetty and Evans, 1980). Cultivars with high HI should be combined with cultivars having high biomass to increase yield (McVetty and Evans, 1980). For Kansas red wheat, the biomass remained same from 1974 to 1987 but grain yield increased 16.2 Kg ha^{-1} yr^{-1} (Cox et al., 1988) probably due to increased HI. Therefore, there is a need for evaluating diverse germplasm for agronomic performance. Eighty two soybean genotypes belonging to MGs III through VIII were evaluated during 1992 and 1993 with the following objectives: (1) To identify genotypes having high biomass, seed yield, and harvest index; (2) To determine variations between maturity groups and among genotypes within a maturity group for biomass, seed yield, and harvest index; and (3) To determine the relationships between seed yield, biomass, and harvest index. The study was part of a regional soybean research project entitled, "Improvement of soybean for stress tolerance and biological efficiency".

Materials and Methods

The experiments were established June 4, 1992 and June 3, 1993 using randomized complete block, with four replications at the Agricultural Research Station, Fort Valley State University. A total of 82 genotypes representing MGs III through VIII were planted. There were eleven genotypes in MG III, twenty two in MG IV, sixteen in MG V, seventeen in MG VI, six in MG VII, and ten in MG VIII. A list of genotypes for each MG is available upon request from the authors. The experimental plots consisted of four 6 m long rows spaced 0.91 m apart. Herbicide Trifluralin [2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzeneamine] was pre-plant soil incorporated @ 1.8 l ha^{-1} during both years. Crop management practices were in accordance with the recommended agronomic practices for soybeans in Georgia. Irrigation was applied as needed at the rate of 25 mm per irrigation by the sprinkler method.

At maturity, plants from one-meter row length were harvested from the middle two rows of each plot for determining biomass and seed yield. Two such samples were taken from each plot and biomass and seed yield were averaged and expressed as Mg ha^{-1}. Harvest index was calculated as the ratio of seed yield to biomass (excluding roots, fallen leaves and debris). All data were subject to statistical analysis using general linear model procedures of SAS Inst. (1988). Genotypic mean separation was by the Duncans Multiple Range Test at 0.05 level of probability. Coefficients of correlation were determined for each MG based on the number of genotypes times replications using the Proc Corr subset of SAS.

Results and Discussion

Biomass

The data on plant biomass, along with three soybean genotypes that showed best performance within MGs III through VIII are presented in Table 1. Significant differences were observed in plant biomass for soybean genotypes in all MGs during 1992 but during 1993, significant differences were observed for MGs IV, VI, and VIII only. Biomass ranged from 2.9 to 10.8 Mg ha^{-1} for MG III during 1992. For both years, the rankings for biomass of three top genotypes, that is, Miles, Century, and Pyramid were similar. In MG IV, biomass ranged from 0.8 to 10.2 Mg ha^{-1} in 1992 but the range was narrow in 1993. The mean biomass for

MG V was 7.8 and 4.5 Mg ha^{-1} in 1992 and 1993, respectively. In MG VI, biomass showed a much wider range in 1992 than that of 1993. In MG V, Vance and L760049 consistently showed top ranking for biomass during both years. The mean biomass for MG VII was 10.9 and 6.5 Mg ha^{-1} during 1992 and 1993, respectively. In MG VII, PI229321 and FC31732 each produced 12.4 Mg ha^{-1} of biomass in 1992. Biomass ranged from 5.2 to 15.5 Mg ha^{-1} for MG VIII during 1992 and 1993. The mean biomass yields for MGs VII and VIII are similar to those reported by Salado-Navarro et al., (1993). The two-year mean biomass yields showed a consistent increase from MG III through VIII, that is, the higher the MG, the higher the biomass. The mean plant biomass for MG VIII was 76.7% more than that for MG III.

Seed yield

Significant variations were observed in yield within MGs and years (Table 2). Seed yield ranged from 1.3 to 5.5 Mg ha^{-1} and 1.5 to 3.7 Mg ha^{-1} for MG III during 1992 and 1993, respectively, with Miles and Pyramid ranking as the two top genotypes. In MG IV, the mean yields were 3.2 and 1.6 Mg ha^{-1} for 1992 and 1993, respectively. During both years, Kent ranked number two for yield.

In MG V, genotype Vance consistently ranked number one in yield and biomass while in MG VI, Davis, Nathan, and Lee yielded more than many other genotypes. The high yield of Miles (MG III) and PI360847 (MG IV) may be attributed to high biomass. Thus, biomass appears to be an important determinant of soybean yield in this study but Salado-Navarro et al., (1993) observed no such relationship. The mean yields of top three genotypes for MGs III, IV, and V were similar (3.7 Mg ha^{-1}). Yield for MG VII ranged from 1.4 to 4.6 Mg ha^{-1}. Similar to their high biomass yields, the two genotypes, PI229321 and FC31732 produced relatively higher yields in MG VII. In MG VIII, Foster showed more stability in yield across years. Among the 82 soybean genotypes examined from MG III to MG VIII, mean yields for MGs VII and VIII were 30% higher than MG III and MG IV.

Harvest index

The mean HI ranged from 42 to 57% for MG III during 1992 (Table 3). Kunitz had the highest HI for both years. The HI in MG IV ranged from 26 to 64% during the two seasons. The HI of MGs VII and VIII ranged from 17 to 47%. Foster, one of the high yielding genotypes in MG VIII, had the highest HI for both years. The mean HI from MG III to MG VIII declined from 47% to about 34%. Again, high yielding genotypes also had high HI. In this study, the mean HI from MGs VI, VII, and VIII was 36% compared to 51% or more reported by Salado-Navarro et al., (1993).

Two cultivars Tokyo (MG VII) and Foster (MG VIII) had high yields as well as high HI during both years, but their biomass yields were only 74 to 83% of their respective MG means. This indicates their superior efficiency in partitioning dry matter into seeds as reported by Gay et al., (1980).

Relationships between biomass, seed yield, and harvest index

The coefficients of correlation (r) among soybean genotypes within MGs for seed yield vs biomass ranged from +0.33 to +0.93 and were significant for both years (Table 4). Similarly r values for seed yield vs HI were significant for all MGs during both years, except for MGs III and VII during 1992. Even though the mean HIs of soybean in MG VII and VIII were low (36% or less), their mean seed yields were similar to those of MGs V and VI, because the genotypes in MGs VII and VIII accumulated more biomass (8.7 to 9.9 Mg ha^{-1}) which could support a greater pod sink size and thus compensated for low HI.

The positive correlations of seed yield with HI and biomass indicate that enhancement of HI and biomass should lead to increase in soybean yields. Our previous research involving a total of over 200 soybean genotypes in 4 field experiments conducted over a period of 6 years showed significant positive correlations (r= 0.22 to 0.78) between seed yield and HI. A higher correlation ranging between 0.59 and 0.92 was observed between seed yield and biomass. These results are consistent with several studies reported for soybean and other crops. Soybean is photoperiod sensitive which complicates the relationships between seed yield, biomass, and HI across environments. Thus, the relationships between biomass, seed yield, and HI would be more consistent within maturity groups compared to between maturity groups. The results of this study and our previous 10 year-data indicate that soybean seed yields can be enhanced by breeding for increased biomass and HI.

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Table 1. Summary of variations among soybean genotypes within maturity groups III through VIII for biomass, 1992-93.

†M G	Range		Mean		Significance		Best performing genotypes	
	1992	1993	1992	1993	1992	1993	1992	1993
	---------- Mg ha^{-1} ----------							
III	2.9 to 10.8	3.6 to 7.5	5.9	5.2	*	NS	Miles (10.8)‡ Century (10.8) Pyramid (8.4)	Miles (7.5) Century (7.3) Pyramid (6.8)
IV	0.8 to 10.2	2.9 to 7.2	6.9	4.6	*	*	Ware (10.2) Custer (10.0) PI360847 (9.3)	PI476880 (7.2) Emerald (6.0) PI248511 (5.9)
V	5.1 to 11.3	3.2 to 5.9	7.8	4.5	*	NS	Vance (11.3)‡ L760049 (10.1) PI398583 (8.9)	Vance (5.9) L760049 (5.6) PI423901-1 (5.1)
VI	5.1 to 17.5	3.6 to 6.6	9.5	5.0	*	*	PI427241 (17.5) Lee (12.6) Davis (12.2)	Davis (6.6) Nathan (6.1) PI423965 (6.0)
VII	7.7 to 13.8	5.1 to 8.9	10.9	6.5	*	NS	PI230970 (13.8)‡ PI229321 (12.4) FC317321 (12.4)	PI229321 (8.9) FC317321 (7.5) Tokyo (7.2)
VIII	9.1 to 15.5	5.2 to 9.4	12.8	6.9	*	*	PI374161 (15.5) PI417136 (15.3) PI247679 (13.8)	Tanbagura (9.4) PI417124 (7.5) Foster (7.3)

†Maturity Group; *Significant at the 0.05 level of probability; ‡Value in parenthesis is the biomass for the corresponding genotype.

Table 2. Summary of variations among soybean genotypes within maturity groups III through VIII for seed yield, 1992-93.

†M G	Range		Mean		Significance		Best performing genotypes	
	1992	1993	1992	1993	1992	1993	1992	1993
	---------- Mg ha^{-1} ----------							
III	1.3 to 5.5	1.5 to 3.7	2.7	2.3	*	*	Miles (5.5) Pyramid (4.3) PI417152 (2.7)	Miles (3.7) Pyramid (3.2) PI464916 (2.6)
IV	0.3 to 6.0	1.1 to 2.6	3.2	1.6	*	*	PI360847 (6.0) Kent (5.1) Custer (4.5)	PI548563 (2.6) Kent (2.0) Emerald (2.0)
V	2.2 to 5.5	1.2 to 2.6	3.8	1.6	*	*	Vance (5.9) PI423827A (4.9) PI398583 (4.5)	Vance (2.6) PI423.901-1 (2.0) PI423827B (1.9)
VI	2.3 to 6.3	1.0 to 2.5	4.1	1.8	*	*	Lee (6.3) Davis (5.3) PI423965 (4.6)	Nathan (2.5) Davis (2.3) Lee (2.2)
VII	2.5 to 4.6	1.4 to 2.8	3.5	2.2	*	*	Tokyo (4.6) PI22932 (3.8) FC31732 (3.7)	Tokyo (2.8) PI22932 (2.8) FC31732 (2.6)
VIII	2.3 to 5.3	1.8 to 3.3	3.8	2.5	*	*	Foster (5.3) Avoyelles (4.8) PI416806 (4.7)	Tanbagura (3.3) Foster (3.1) PI417124 (3.0)

†Maturity Group; *Significant at the 0.05 level of probability; ‡Value in parenthesis is the seed yield for the corresponding genotype.

Table 3. Summary of variations among soybean genotypes within maturity groups III through VIII for harvest index, 1992-93.

†M G	Range		Mean		Significance		Best performing genotypes	
	1992	1993	1992	1993	1992	1993	1992	1993
	---------- % ----------							
III	42 to 57	40 to 51	49	45	*	*	Kunitz (57) PI464916 (53) Miles (52)	Kunitz (51) PI417303 (50) PI464916 (48)
IV	35 to 64	26 to 46	47	36	*	*	PI360847 (64) Douglas (56) Sango (56)	Kent (46) PI82291 (45) Sango (45)
V	32 to 56	27 to 45	48	37	*	*	PI423827A (56) PI407907A (55) PI43901-1 (54)	PI423827A (45) PI398583 (45) Vance (44)
VI	26 to 58	24 to 41	45	34	*	*	PI423965 (58) PI416937 (53) Lee (50)	Nathan (41) PI398479 (40) Lee (39)
VII	27 to 47	27 to 38	35	33	*	*	Tokyo (47) FC31927 (40) PI230972 (36)	Tokyo (38) FC31732 (36) PI230972 (35)
VIII	17 to 42	29 to 42	31	36	*	*	Foster (42) PI417124 (42) Avoyelles (35)	Foster (42) Bryan (41) PI417124 (40)

†Maturity Group; *Significant at the 0.05 level of probability; ‡Value in parenthesis is the harvest index for the corresponding genotype.

Table 4. Coefficients of correlation (r) between soybean seed yield, biomass, and harvest index during 1992 & 1993.

Parameter	N	Coefficients of correlation (r)		
		1992	1993	†Combined 1992 & 1993
Seed yield vs. Biomass :				
Maturity Group III	44	0.62***	0.83***	0.67***
IV	88	0.82***	0.74***	0.84***
V	64	0.75***	0.69***	0.87***
VI	68	0.52***	0.85***	0.72***
VII	24	0.64***	0.93***	0.77***
VIII	36	0.33*	0.83***	0.61***
Maturity Groups combined	**322**	**0.60*** **	**0.83*** **	**0.73*** **
Seed yield vs. Harvest index:				
Maturity Group III	44	NS	0.51***	0.37***
IV	88	0.44***	0.43***	0.59***
V	64	0.54***	0.49***	0.68***
VI	68	0.45***	0.72***	0.65***
VII	24	NS	0.48**	NS
VIII	36	0.73***	0.46**	0.41***
Maturity Groups combined	**322**	**0.31***	**0.45*** **	**0.47*** **

*,**,& *** Significant at the 0.05, 0.01, & 0.001 level of probability, respectively. †N equals twice that shown in column two.

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A Modified Procedure for Mitotic Chromosome Count in Soybean.

The soybean has 2n=40 small somatic chromosomes. A better and reliable procedure for mitotic study is useful in cytogenetic analysis in soybean. We report a staining procedure of soybean mitotic chromosomes, which is better than those reported earlier (Singh, 1993).

Mitotic chromosomes were examined as follows. The soybean seeds were germinated in a sand bench in the greenhouse. Root-tips from actively growing 7 to 10 d-old-seedlings were collected in 1.5 ml microcentrifuge tubes containing double distilled water. Root-tips were pretreated with 0.05% 8-hydroxyquinoline for 4-5 h at 16°C in a Micro Cooler 03oekel Industries, Inc.; 509 Vine Street, Philadelphia; model 260011). Pre-treated Root-tips were fixed in a 3:1 (v/v) mixture of 95% ethanol and propionic acid for 24 h. The fixative were removed from the microcentrifuge tubes, and the root-tips were washed once with double distilled water. Root-tips were hydrolyzed in 1*M*HCl for 11-15 min at 60°C. After hydrolysis, the root-tips were rinsed in double distilled water and stained in Schiff's reagent (Fuchsin-sulphate reagent, Sigma lot# 51H5014) for 2 to 4 h at room temperature in the dark. Feulgen stain was removed and the root-tips were rinsed with cold double distilled water and stained with Carbol fuchsin stain overnight at O°C - 4°C in a refrigerator. After Carbol fuchsin staining, the root-tips were washed three to four times with cold double distilled water and stored in cold double distilled water in a refrigerator. Root-tips were squashed in 45% acetic acid under a clean cover glass.

The Carbol fuchsin stain used in this study was slightly modified from that reported by Darlington and LaCour (1975) and Xu and Joppa (1995). The stain was made up in the following three consecutive solutions: Solution A was made up by mixing 10 ml 3% basic fuchsin (in 70% ethanol) with 90 ml 5% phenol; solution B by mixing 55 ml solution A, 6 ml glacial acetic acid, and 3 ml formalin; and the Carbol fuchsin staining solution by mixing 20 ml solution B and 80 ml 45% acetic acid plus 1.8 g sorbitol. We have found the cytological technique for mitotic chromosomes described here to be the best for soybean chromosome preparations because this procedure yields a large number of cells with metaphase chromosomes. The staining of chromosomes is optimal, the cytoplasm is clear and the chromosomes are well spread for precise counting.

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A Plant Transformation Ready Bacterial Artificial Chromosome Library for Soybean: Applications in Chromosome Walking and Genome Wide Physical Mapping.

Introduction

The soybean [Glycine max (L.) Merr.] cultivar 'Forrest' is a source of resistance genes to both soybean cyst nematode (SCN) and sudden death syndrome (SDS) caused by *Heterodera glycines* and sudden death syndrome caused by *Fusarium solani* f. sp. glycines.

We have constructed a high-resolution genetic map of the two loci *Rhg4* and *rhg1* conferring resistance to SCN race 3. Linked to *rhg1* we have detected two genes *rfs1* (3 cM away) and *rft1* (<0.25 cM away) that confer resistance to SDS (Meksem et al., 1998). Several other agronomically important genes (disease resistance, yield, resistance to manganese toxicity, drought tolerance) have been mapped as QTL in the cultivar Forrest (Chang et al., 1997).

We have developed two new bacterial artificial chromosome (BAC) vectors that will accelerate the cloning of *Rhg4*, the *rhg1, rfs1, rft1* gene cluster and other agronomically important genes. These vectors allow large DNA fragments to be used transform plants directly instead of after subcloning of small fragments. The vector V41 (Zhang et al., 1998) is similar to pBIBAC1, (Hamilton et al., 1996) but with *lacZ* based color selection for inserts, tetracyclin resistance for bacterial propagation and lacking hygromycin resistance for selection in plants.

We have constructed a Forrest Bacterial Artificial Chromosome (BAC) library in the V41 vector (9.5X haploid genome equivalent with an average insert size of 125 Kb). This library will provide clones for physical mapping of the soybean genome and for chromosome walking or landing. Genetic complementation tests in transformed plants with candidate clones containing target genes can be made directly via Agrobacterium-mediated methods. Using the Forrest BAC library, we developed a new method to isolate microsattelites DNA markers for gene cloning and marker assisted selection.

Genome wide physical mapping with BACs by fingerprinting and contiguous clone assembly technology, was adopted in our lab. (Zhang et al., 1997) for rapidly cloning of soybean agronomic genes and QTL.

Materials and Methods

Plant materials

The soybean [Glycine max (L.) Merr] cultivar Forrest (Hartwig and Epps, 1973) was used to construct the *Bam*HI and the *Hind*III BAC libraries.

Vector

The BAC vector V41 was kindly provided by Dr. H. Zhang, Texas A & M University, USA.

DNA probes and primers

The Bng122 RFLP probe was provided kindly by Dr. E. Vallejos (University of Florida, USA). The microsatellite primers (BARC-SATT 309, BARC-SATT 275, BARC-SATT 163, BARC-SATT 214) for screening the BAC library were provided kindly by Dr. P. Cregan (USDA, Beltsville, MD, USA). The microsatellite primers for SIUC-SAT122 were generated in this study and used for screening the BAC library. The BLT65 primers were provided by Dr. Ben Matthews (USDA, Beltsville MD, USA).

Preparation and digest of high molecular weight DNA:

The method for HMW DNA preparation from plant nuclei is described by Zhang et al. (1994). Two to three weeks after germination in the greenhouse seedlings were transferred into dark to reduce carbohydrate. Nuclei were prepared from 25 g of leaves embedded in 12 ml of low melting point agarose microbeads. The partial digestion of the microbeads (800 µl = ~5 µg DNA) was performed by fixing the time of digestion to 10 min at 37 C and varying the amount of the enzyme *Bam*HI (*Hind*III) from 0.1-2 unit (Woo et al., 1995).

Construction, storage and analysis of the BAC library

The vector V41 (Zhang et al., 1998) was digested with *Bam*HI (*Hind*III) and then dephosphorylated to prevent self ligation (Woo et al., 1994). Partially digested HMW DNA was size selected by PFGE on a CHEF DRIII (Bio-Rad, Hercules, CA; Woo et al., 1994). Three size selections were performed to increase the average insert size of BAC's, and to eliminate small DNA fragments trapped in the HMW fraction.

The HMW genomic DNA and the vector DNA were ligated and electroporated into *E-coli* ElectroMAX DH10B strain (Gibco-BRL, MD). Recombinant transformants were selected on media containing tetracycline, IPTG and X-gal and individually picked and stored into 384 well plates at -80 C (Zhang et al. 1996). The average insert size was assayed by a simple alkaline lysis DNA minipreparation (Sambrook et al., 1989) followed by restriction digest with *Not*I to free the DNA insert from the vector, and PFGE (Frijters et al .,1997).

BAC library screening

A Beckman 2000 Automated robot was used to spot the clones of the BAC library onto Hybond N+ filters of 12 x 8 cm each containing a duplication of each colony of four 384-well microtiter plates. The entire library of 38400 *Bam*HI and 38400 *Hind*III BAC clones was inoculated onto 50 filters. The inoculated filters were placed on lids of 96-well microtiter plates containing LB agar and

25 µg/ml tetracycline and incubated at 37 C overnight. When the colonies reached a size of 2-3 mm in diameter, the filters were processed according to Zhang et al. (1996) followed by hybridization to random hexamer 32P-labeled DNA probes (Sambrook et al., 1992).

Pools and super-pools of the BAC library

Pools and super-pools of DNA of 384 and 1536 BAC were constructed by replicating each 384-well microtiter plates in the library to a plate containing LB agar and 25 µg tetracycline. After overnight incubation at 37 C, cells from each plate were harvested (pooled) with 5 ml LB into one tube. Plasmid DNAs isolated by alkaline lysis procedure (Sambrook et al., 1989) were stored in microfuge tubes. Superpools were constituted from DNA of one 384-well plates and pools from DNA of four 384-well plates.

AFLP fingerprinting of BACs

BAC DNA was analyzed by AFLP fingerprinting using various *Eco*RI and *Mse*I primer combinations that identified tightly linked AFLP markers to *rhg1* and *Rhg4* locus as described by Simons et al. (1997)

Microsattelite markers screening of the BAC library

The microsattelites primers were labeled each by phosphorylating the 5' end with 5µl [γ-^{32}P] ATP (3000 Ci/mmol) for 30 min at 37 C with 10 Unit of T4 Kinase (Pharmacia, Piscataway, NJ). Standard microsattelite PCR reaction (Akkaya, et al. 1992) were performed with pools and subpools of BAC library DNA.

Fingerprinting of BACs for physical mapping

The BAC clones DNA was first prepared by alkaline lysis, the fingerprint was performed as described in Tao et al. (Patent pending), fingerprinted DNA was fractionated on 4% (w/v) denaturing polyacrylamide gel.

Results

Construction of the Forrest BAC libraries

About 0.4 ng/µl of size- selected DNA fragments was harvested after the third size selection. Approximately 5,000 to 6,000 recombinants clones were obtained from each single electroporation and 10,000 to 15,000 recombinant clones from each nanogram of ligated DNA. Two soybean Forrest BAC libraries were constructed, one using *Bam*HI and the other *Hind*III partially restricted fragments, both contained 38,400 clones each.

Characterization of the forrest BAC libraries

To estimate the average insert size and insert size distribution of the *Bam*HI and *Hind*III BAC libraries, DNA of 100 random clones from each *Bam*HI and *Hind*III BAC library were isolated, digested with *Not*I to release the inserts from the vector and analyzed by PFGE (Fig. 1). The insert size of each clone was determined by adding the sizes of all fragments in each lane except for the common BAC vector bands. The clones were grouped by insert size and the insert size was plotted versus the frequency of each group of clones present in the library (Fig. 2). The two BAC libraries have an average insert size of 125 Kb, which over 76,800 clones is equivalent to 9.5 haploid genomes of soybean. The insert sizes of the clones in those libraries varies from 60 to 260 Kb and 65% of the clones have insert larger than 100 Kb. Theoretically, the probability of obtaining a particular clone from this libraries is over 99%.

Fingerprinting of BACs for soybean physical mapping

The first fingerprint experiment shows that 25 to 50 fragments can be generated from a single BAC fingerprint, every single BAC fingerprint profile is different to the next (Fig.3). Common bands can be observed between clones isolated by microsatellite screening of the BAC library (lanes 31-39 and 41-49). Individual clones will be used to nucleate contigs.

Discussion

The average DNA insert size of the *Bam*HI and *Hind*III Forrest BAC libraries are comparable to the majority of plant BAC libraries (Woo et al., 1994; Wang et al., 1995; Xiaozhu et al., 1995; Danesh et al., 1996; Zhang et al., 1997). The combined *Bam*HI and *Hind*III Forrest BAC libraries represent 9.5 haploid equivalents of the soybean genome . Therefore, there is a 99% chance of obtaining one specific BAC clone during library screening with a specific sequence. This has been confirmed during the screening of the BAC library with the microsattelite primers for SATT309, SATT275, SATT214, SIUCSAT122 and SATT163, and the RFLP probe Bng122, and will be further confirmed by more screening. The genetic distance between the Bng122 marker and the SIUCSAT122 marker was 1 cM, corresponding to approximately 110 Kb. This is in close agreement with a ratio of physical to genetic distance of about 100 Kb/cM for this interval (Danesh et al., 1996).

BAC libraries constructed with more than one restriction enzyme are more suitable for assembling long continuous contigs than a single library constructed with one enzyme. The two soybean BAC libraries were constructed from Forrest, parent of a mapping population consisting of 100 F5:9 RILs and 100 RIL-derived NIL populations containing 40-4,500 NILs which segregate for many traits of agronomic importance. The combined RIL and NIL mapping populations should provide an invaluable framework for soybean genome research. The BAC libraries constructed from one parent of such widely available RIL mapping population will provide a common framework for physical mapping of the soybean genome.

Target genes and the linked RFLP, microsattelite markers and markers isolated from a chromosome walking experiment can be more accurately mapped using this population and the physical map. Therefore, the BAC libraries will be readily used to clone the target genes by map based cloning and assemble long rang overlapping contigs.

Bac fingerprinting for gene golfing

Data generated by the new fingerprint approach (Tao et al. Patent pending) were of higher quality than those used to construct the yeast, *C.elegans* and human physical maps (Coulson et al., 1986; Wong et al., 1997 and Marra et al., 1997). The fingerprint method allows contig assembly across

centromeric repeat regions and can distinguish homeologous sub-genomes in diploid, (Tao and Zhang, unpublished) and diploidized tetraploid species (Meksem et al., unpublished). These features are essential for contig construction in complex genomes.

BAC clones recovered in chromosome landing and walking experiments were characterized by fingerprinting and the fingerprints were used to build contigs on LG G (Figure 3, lanes 31-39) and LG A2 (Figure 3, lanes 41-49). In addition, the Forrest soybean BAC library was screened with a high copy RFLP probe [A071] and 57 positive clones were identified. Among these six separate groups could be identified that each contained common bands significant for contig construction and to distinguish the homeologous loci (not shown). A similar approach is being used with centromere repeat containing BAC clones to prove the method can create physical maps through soybean centromeric regions as through rice and *Arabidopsis* centromeric repeats.

Advantages offered by this approach include data that are comparatively free of artifacts, compatibility with pre-existing software developed at the Sanger Center, and the high throughput necessary to fuel our genome study goals. Integration with the microsatellite genetic map will allow the soybean genetics community to rapidly isolate genes underlying traits of agricultural importance.

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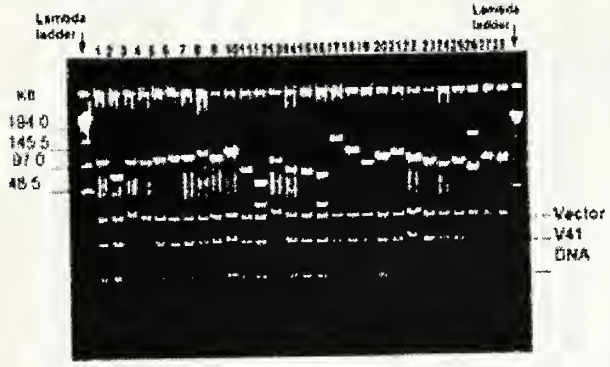
Figure1: *PFGE analysis of BamHI and HindIII Bac's containing soybean DNA from Forrest cultivar*



Bac clones analysis: DNA was isolated from randomly-selected BAC clones by alkaline miniprepparation, digested by *MseI*. The gel was stained in ethidium bromide after PFGE

Figure 2:

Size distribution of BAC clones randomly taken from the Forrest BAC library

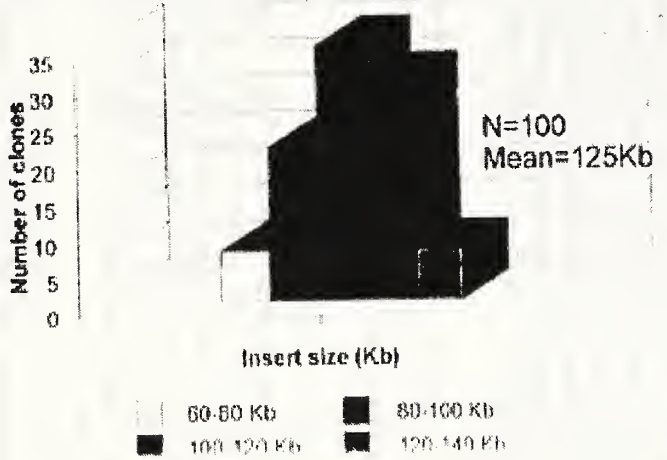
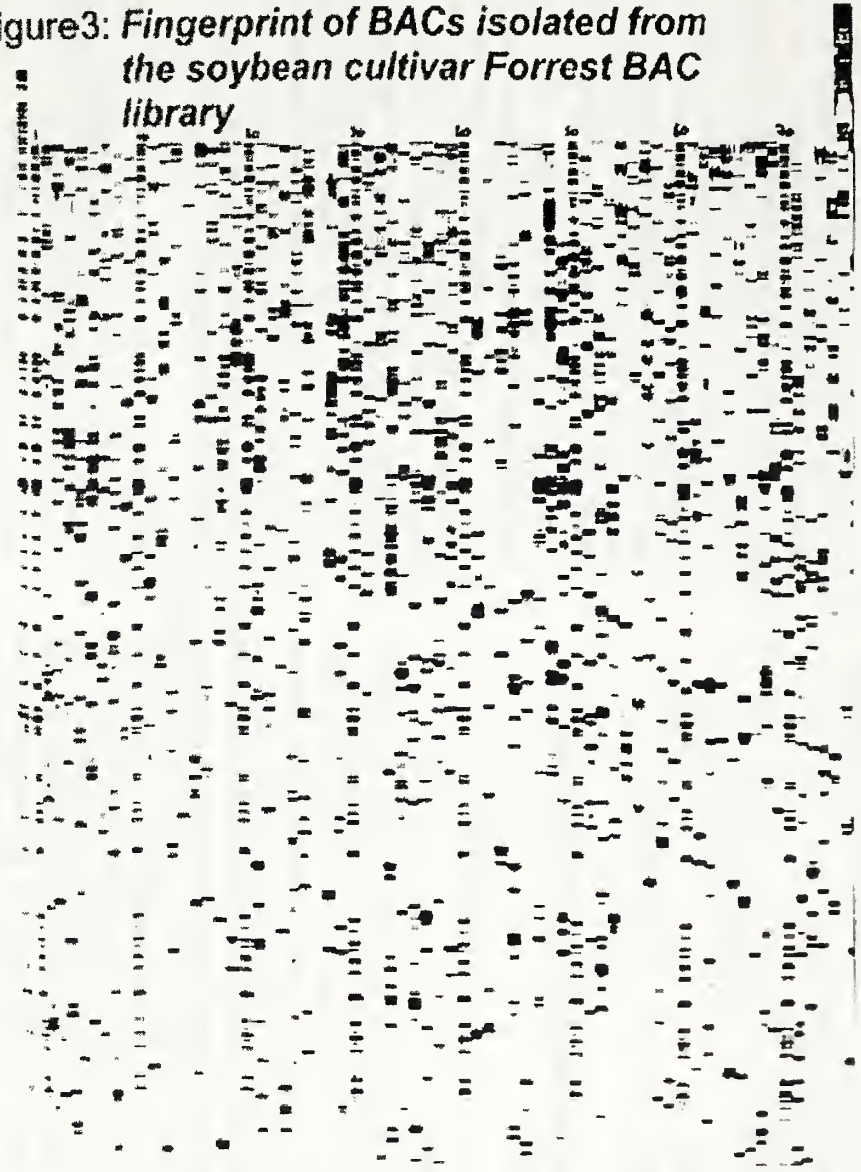


Figure3: ***Fingerprint of BACs isolated from the soybean cultivar Forrest BAC library***



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Apparent Source of the *Rj5* Allele in Soybean Cultivars

Introduction

In soybean (*Glycine max*. (L.) Merr.), there are three dominant genes, *Rj2* (Caldwell, 1966), *Rj3* (Vest, 1970), and *Rj4* (Vest and Caldwell, 1972) associated with an ineffective nodulation response with specific strains of *Bradyrhizobium japonicum* (Kirchner) Buchanan and three recessive genes, *rj1* (Williams and Lynch, 1954), *rj5* and *rj6* (Pracht et al. 1993), associated with a non-nodulating response when inoculated with most strains of *B. japonicum*. Recessive genes, *rj5* and *rj6*, were identified (Pracht et al. 1993) in a non-nodulating mutant (NN5) isolated from N-nitroso-N-methylurea mutagenized 'Williams' seed (Ryan and Harper, 1983). When both *rj5* and *rj6* genes are present, roots of NN5 fail to nodulate even when challenged with high inoculum rates (10^9 to 10^{11} cells) of *B. japonicum* strains; rates that do result in sparse nodulation of *rj1* non-nodulating lines (Francisco and Harper 1994). Thus, non-nodulation control by *rj5* and *rj6* (NN5 mutant) is more restricted than by *rj1*. From studies by Pracht et al. (1993), it was concluded that Williams, which was the source of the NN5 mutant, carries *rj5* and *Rj6*. The mutation resulted in the expression of *rj6* in the NN5 line. It was also established by Pracht et al. (1993) that Harosoy 63 carries *Rj5* and *Rj6*.

The objective of this study was to determine the source and origin of *Rj5* in Harosoy 63 and to determine the presence of *Rj5* in twenty-six other normal nodulating cultivars.

Materials and Methods

To test if the *Rj5* allele is present, NN5 (non-nodulating mutant) was used as a tester to classify nodulated cultivars. Crosses were made at the University of Illinois between NN5 and several nodulating cultivars, including the ancestor lines of Harosoy 63; A.K. (Harrow), Mandarin (Ottawa), and Blackhawk (Table 1). The F_1 seeds were planted in the field and F_2 seeds were harvested in the fall.

The F_2 seeds were inoculated with commercial peat-based *Bradyrhizobium japonicum* (Urbana Laboratories, St. Joseph, MO) and planted in the greenhouse in 10 cm pots filled with sand. Approximately ninety seeds of each cross were planted and visually evaluated 21 days after emergence. Roots of the individual F_2 plant were classified as either nodulating or non-nodulating. Chi-square tests were used to measure goodness of fit to expected ratios.

Results and Discussion

Of the 26 nodulated soybean lines tested in crosses with the NN5 non-nodulated line (Table 2), 13 lines apparently carry the *Rj5* gene as evidenced by the F_2 segregation ratio of fifteen normally nodulated: one non-nodulated, as established by Pracht et al. (1993). The other 13 lines fit a three normally nodulated: one non-nodulated ratio, indicating that *rj5* was present. No pattern was evident for a specific geographical origin of lines which gave rise to the *rj5* gene since lines from China, South Korea, Japan, Russian Federation, and Taiwan were found to express *rj5*.

Ancestor lines of Harosoy 63 (Table 1) were evaluated to allow identification of the source of the *Rj5* gene. Data from F_2 plants from the cross between NN5 and Blackhawk, and the combined data from the reciprocal crosses of NN5 with Mandarin (Ottawa) segregated three normal nodulating: one non-nodulating, indicating that these cultivars contain the recessive *rj5* gene (Table 2). In contrast, F_2 plants from the cross between NN5 and A.K. (Harrow) segregated in a theoretical ratio of 15 normal nodulating: one non-nodulating indicating that *Rj5* was present (Table 2). From this study it was concluded that the dominant *Rj5* gene in Harosoy 63 came from A.K. (Harrow) and is responsible for normal nodulation.

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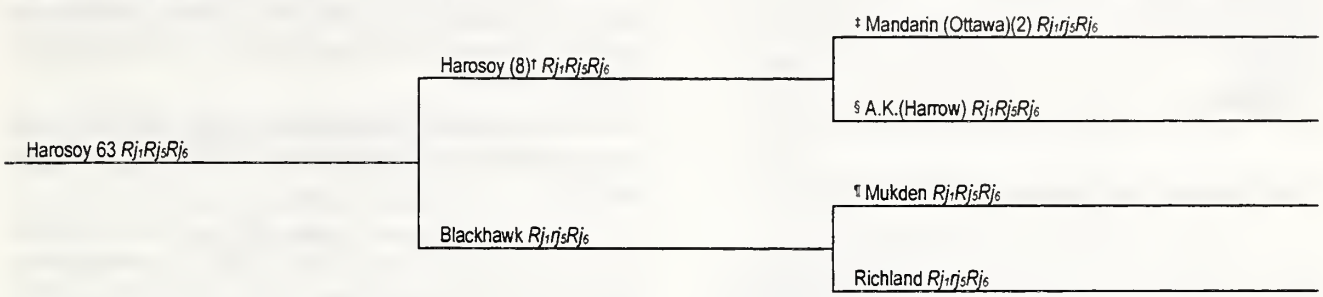

† Number in a parenthesis indicates the number of backcrosses.
‡ Mandarin(Ottawa) was selected from Mandarin that was introduced from Heilongjiang, China
§ A. K. (Harrow) was selected from A. K. that was introduced from northeast China.
¶ Mukden introduced from Liaoning, China.
Richland introduced from Jilin, China

Table 2. The origin of nodulating soybean breeding lines and cultivars tested for *Rj*₅ using segregation among F_2 plants from crosses of the non-nodulating mutant of Williams, NN5, in response to inoculation with *Bradyrhizobium japonicum*.

		Observed				
Nodulating parent	Source	Nodulating	Non-nodulating	Total plants	X^2	P†
Theoretical 3:1 suggesting *rj*₅ is present.						
Mandarin‡	Heilongjiang, China	135	52	187	0.79	0.38
Richland‡	Jilin, China	66	16	82	1.32	0.25
Blackhawk‡	Mukden‡ x Richland‡	58	25	83	1.16	0.28
Edison§	HW79116 x HW79022	63	19	82	0.15	0.70
Spry¶	L78-8694 x L78L-449	56	28	84	3.11	0.08
IA3003	Chamberlain‡ x Conrad#	58	20	78	0.02	0.90
HS88-4906	Conrad# x Hayes††	54	22	76	0.63	0.43
IA2007	Pride B152‡‡ x A80-244003§§	61	15	76	1.12	0.29
LN88-9242	Sherman‡ x Chamberlain‡	61	18	79	0.21	0.65
PI 407788A	South Korea	50	32	82	8.60	0.00
PI 423948A	Japan	62	16	78	0.84	0.36
PI 437088A	Russian Federation	38	21	59	3.53	0.06
PI 518758	Taiwan	50	11	61	1.58	0.21
Theoretical 15:1 suggesting *Rj*₅ is present.						
A. K. (Harrow)‡	Selected from A. K.	150	18	168	5.71	0.02
Mukden‡	Liaoning, China	72	4	76	0.13	0.72
Century 84‡	Calland‡ x Bonus‡	65	5	70	0.10	0.76
LL89-605¶¶	Wells II‡ x PI 437821	67	6	73	0.48	0.49
LN88-7616	Hack‡ x HW8221##	72	8	80	1.92	0.17
Iroquois†††	LN81-1029 x Asgrow A2943	68	3	71	0.50	0.48
LN89-5322-2‡‡‡	(Sherman x Asgrow A2943) x Elgin	68	6	74	0.44	0.51
PI 417163	Japan	78	6	84	0.11	0.74
PI 417468	Japan	35	4	39	1.07	0.30
PI 437716A	China	72	6	78	0.28	0.60
PI 437789	China	82	8	90	1.07	0.30
PI 438495	U.S. (Laredo)	65	7	72	1.48	0.22
PI 475824A	Xinjiang, China	48	4	52	0.19	0.67

† Probability of obtaining a higher value of chi-square by chance alone.
‡ Bernard et al., 1988.
§ McBlain et al., 1991b.
¶ Bernard and Nickell, 1992.
# Fehr et al., 1988.
†† McBlain et al., 1991a.
‡‡ Pride B152 = [(Roanoke x Wayne) x Pella. (Bernard et al., 1988).
§§ A80-244003 = (Corsoy x Wayne) x Pella. (Bernard et al., 1988) Nickell et al., 1996.
¶¶ Nickell et al., 1994.
HW8221 = AP6 x (Tracy x Williams). (Fehr and Ortiz, 1975, Bernard et al., 1988).
††† Nickell et al., 1996.
‡‡‡ Stephens and Nickell, 1992.

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Biosystematics of the Genus Glycine, 1997

Table 1 contains a list of the wild perennial Glycine species in the subgenus Glycine and the annual species in the subgenus Soja. In addition, for each species there is a three letter identification code, 2n chromosome number, cytological standard, 1L number, PI number, genome symbol and distribution. The main points are as follows:

1. Currently there are 16 wild perennial Glycine species. All carry 2n = 40 chromosomes except HIR, TAB, and TOM. These species also have polyploid cytotypes. In addition, TOM has 2n = 38 and 78 cytotypes. The genus Glycine in an ancient polyploid having x = 10. However, the 2n = 40 plants behave cytologically like diploids. The annual Glycine, SOJ and MAX were derived from the perennial forms. Glycine soja is the ancestor of G. max, the cultigen.

2. The annual Glycine are indigenous to Asia while the wild perennial Glycine are indigenous to the Australian tectonic plate. Seeds of wild perennial species found outside the Australian plate were brought to these regions by migratory birds via long distance dispersal.

3. Species relationships via genome symbol designations has been elucidated by cytogenetic, biochemical, and molecular approaches.

4. Recently, a new species, Glycine dolichocarpa was described from Taiwan by Tateishi and Ohashi (1992). Examination of live plants in the field and subsequent cytological studies at Illinois, suggest that the plants are members of the G. tomentella 80 chromosome allopolyploid species complex.

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Table 1. List of the species in the genus *Glycine* Willd., three letter code, 2*n*, PI number, genome symbol, and distribution.

Species	Code	2n	Standard-PI	Genome	Distribution
Subgenus *Glycine*					
G. albicans Tind. & Craven	ALB	40	—	II	Australia
G. arenaria Tind.	ARE	40	505204	HH	Australia
G. argyrea Tind.	ARG	40	505151	A_2A_2	Australia
G. canescens F.J. Herm.	CAN	40	440932	AA	Australia
G. clandestine Wendl.	CLA	40	440958	A_1A_1	Australia
G. curvata Tind.	CUR	40	505166	C_1C_1	Australia
G. cyrtoloba Tind.	CYR	40	440962	CC	Australia
G. falcata Benth.	FLA	40	505179	FF	Australia
G. hirticaulis Tind. & Craven	HIR	40, 80	—	H_1H_1	Australia
G. lactovirens Tind. & Craven	LAC	40	—	I_1I_1	Australia
G. latifolia (Benth.) Newell & Hymowitz	LAT	40	378709	B_1B_1	Australia
G. latrobeana (Meissn.) Benth.	LTR	40	483196	A_3A_3	Australia
G. microphylla (Benth.) Tind.	MIC	40	440956	BB	Australia
G. pindanica Tind. & Craven	PIN	40	—	H_2H_2	Australia
G. tabacina (Labill.) Benth.	TAB	40	373990	B_2B_2	Australia
	TAB	80	—	Complex[1]	Australia, West Central and South Pacific Islands
G. tomentella Hayata	TOM	38	440998	EE	Australia
	TOM	40	505222	DD[2]	Australia, Papua New Guinea
	TOM	78	—	Complex[3]	Australia, Papua New Guinea
	TOM	80	—	Complex[4]	Australia, Papua New Guinea, Philippines, Taiwan
Subgenus *Soja* (Moench) F.J. Herm.					
G. soja Sieb. & Zucc.	SOJ	40	81762	GG	China, Russia, Taiwan, Japan, Korea Cultigen
G. max (L.) Merr.	MAX	40	—	GG	

[1] Allopolypoids (A and B genome) and segmental allopolyploids (B genome); [2] At least four genomic groups;
[3] Allopolyploids (D and E, A and E, or any other unknown combination); [4] Allopolyploids (A and D genomes, or any other unknown combination).

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Biotechnology approaches to Improving Resistance to SCN and SDS: Methods for high throughput Marker Assisted Selection.

Introduction

The soybean [Glycine max (L.) Merr.] cultivars such as 'Forrest' "Hartwig' and 'Jack are sources of resistance genes to both soybean cyst nematode (SCN) and sudden death syndrome (SDS) caused by *Heterodera glycines* and sudden death syndrome caused by *Fusarium solani* f. sp. glycines (Chang et al., 1997; Meksem et al., 1998). We have developed methods for DNA marker assisted selection (MAS) to recover resistance to both pathogens. The DNA markers used are products of several programs throughout the US (Akkaya et al., 1995). The methods for their use with selected and nonselected soybean populations, using leaf or seed fragments have been developed and tested at SIUC. The methods detailed herein were presented at a SCN Workshop following the Soybean Breeders Meeting on February 26, 1998. We used the methods to screen 10,000 genotypes in 1997 and plan to screen 100,000 in 1998.

Materials and Methods

DNA probes and primers.

The microsatellite primers (BARC-SATT 309, BARC-SATT 275, BARC-SATT 163, BARC-SATT 214) for screening were provided kindly by Dr. P. Cregan (USDA, Beltsville, MD, USA). The microsatellite primers for SIUC-SAT122 were generated in this study and used for screening the BAC library. The BLT65 primers were provided by Dr. Ben Matthews (USDA, Beltsville MD, USA).

DNA extraction methods from leaves:

1. Pick the youngest leaves off of each plant (this procedure will not work on old leaves).
2. Cut a circle out of one leaf with the back of a 200 µL pipette tip.
3. Place the leaf circle in a microfuge tube or a microtiter plate.
4. Immediately before grinding, add NaOH (50 µL; 0.5 M).
5. Grind by hand with a plastic pestle or grind in a Matrix Mill.
6. Dilute sample (10 µL) into Tris (90 µL; 100 mM; pH 8.0)

Nondestructive DNA extraction methods from seed:

1. Cut the seed (approx. 15% _ 0.15 g) opposite the root tip with a scalpel.
2. Scrape off the seed coat with a scalpel.
3. Soak the seed slice in SDS (2%; 400 µL) overnight (R.T.) in a microtiter plate.
4. Add PVP powder (1/2 weight; approx. 0.08 g) to each well.
5. Crush the seed with a fitted plastic pestle.
6. Let the polysaccharides settle out (R.T.; 15 min.).
7. Dilute the sample (10 µL) into Tris (90 µL; 100 mM; pH 8.0).

Amplification:

1. Create a master mix of the ingredients below by multiplying the number of samples by the amount needed for each ingredient. An example is shown.

1a. Always make enough mix for a few extra samples.

1b. Add the Mg^{2+} and the polymerase last.

2. Add the master mix to every PCR tube.
3. Add template DNA to the tubes and mix them by pipetting 3-5 times.
4. Add oil to the top of each tube if a heat lid will not be used.
5. Set the program below.

Satt 309

	Buffer	Mg^{2+}	dNTP	F. Primer	R. Primer	Polymerase	Water	Template
	2.5	2.5	2.5	0.5	0.5	.3	11.2	5.0
50X	125	125	125	25	25	15	560	-
Conc.	1X	2.5 mM	200 µM	100 nM	100 nM	1.5 units		

Satt 309

	Denaturation	Ramp	Annealing	Ramp	Extension	Cycles
	94° C		47° C		72° C	
Stage 1	2 min.					1
Stage 2	30 sec.	2 min.	30 sec.	–	1:30 min.	32
Stage 3					5 min.	1

-The products are both around 200 bps long, so they must be visualized on high purity agarose or polyacrylimide.

Satt 38

	Buffer	Mg^{2+}	dNTP	F. Primer	R. Primer	Polymerase	Water	Template
	2.5	1.5	2.5	0.5	0.5	.3	11.2	5.0
50X	125	75	125	25	25	15	610	-
Conc.	1X	1.5 mM	200 µM	100 nM	100 nM	1.5 units		

Satt 38

	Denaturation	Ramp	Annealing	Ramp	Extension	Cycles
	94° C		47° C		72° C	
Stage 1	2 min.					1
Stage 2	30 sec.	2 min.	30 sec.	--	1:30 min.	32
Stage 3					5 min.	1

-The products are both around 200 bps long, so they must be visualized on high purity agarose or polyacrylimide.

Blt 65

	Buffer	Mg^{2+}	dNTP	F. Primer	R. Primer	Polymerase	Water	Template
	2.5	2.5	2.5	1.0	1.0	.2	11.2	5.0
50X	150	150	150	50	50	10	515	-
Conc.	1X	2.5 mM	200 µM	400 nM	400 nM	1.0 unit		

Blt 65

	Denaturation	Ramp	Annealing	Ramp	Extension	Cycles
	94° C		52° C (55)		72° C	
Stage 1	4 min.					1
Stage 2	30 sec.	2 min.	30 sec.	--	1:30 min.	35
Stage 3					5 min.	1

-The products are around 500 bps long, and they can be visualized on a 1.4% electrophoresis grade agarose gel.

Product visualization:

1.4% Electrophoresis Grade agarose:
This example if for a 180 mL gel.

1. Measure out TAE buffer (1X; 180 mL).
2. Weigh agarose (2.52 g; electrophoresis grade).
3. Pour the agarose into the buffer and mix thoroughly (by swirling flask).
4. Heat in the microwave (high) and stir (every 30 sec.).
5. Remove the agarose when it boils and mix it.
6. Heat until it boils again.
7. Let it cool while slowly stirring it with a glass stir rod.
8. Add ethidium bromide (10 mL; 1.0 mg/mL) anytime before pouring gel.

8a. •Use CAUTION when handling ethidium bromide.
8b. •Double glove.
8c. •Dispose of ethidium bromide stained items as hazardous waste.
8d. •Do not use a stir rod after the stain has been added.

9. Pour the gel when the temperature is 50-60° C.
10. Let cool until solid and opaque (30 min.).
11. Add gel running dye (1/5 volume) to each sample before loading them into the wells of the gel.
12. Run the gel at 80-120 volts (40 min.).
13. Visualize the gel under UV illumination (320 nm).

13a. •Wear a face shield when looking at UV light.

14. Dispose of the gel in a hazardous waste container.
15. A double band around 500 bps is the Forrest SDS resistant allele.

15a. A single band around 500 bps in the Essex SDS susceptible allele.

(TAE Buffer (50X) is - 57 mL acetic acid, 242 g Tris base, and 100 mL EDTA pH 8.0 into water to make 1 Liter. Gel Running Dye - 20% (w/v) Fucol, 1% (w/v) bromophenyl blue, water.

4% Metaphor agarose (FMC BioProducts, Rockland, ME):
This example is for a 180 mL gel

1. Chill TBE buffer in the refrigerator (180 mL; 1 hour).
2. Weigh Metaphor agarose (7.2 g).
3. Slowly add the agarose to the chilled buffer while stirring.
4. Let the mixture sit (15 min.; R.T.).
5. Cover the beaker with plastic wrap and pierce it.
6. Heat in the microwave on medium (2 min.).
7. Slowly swirl the beaker to resuspend any agarose on the bottom.
8. Heat on high until the solution boils (mixing every 30 sec.).
9. Remove from the microwave when all of the gel pieces have dissolved.
10. Gently swirl the beaker to mix everything.
11. Boil one more time.
12. Use a glass rod to stir the agarose while it cools.

12a. If agarose sticks to the rod, wipe the rod on a paper towel.
12b. Try to remove any big lumps with the glass rod.
12c. Don't worry if there are some left.

13. Pour the agarose into a gel tray when it reaches 50-60°C
14. Let it cool until solid (30-40 min.).
15. Refrigerate the gel (20 min.) prior to its first use.
16. Stain the gel in dilute ethidium bromide (0.1-1 mg/mL; 10 min.).

16a. •Use CAUTION when handling ethidium bromide.
16b. •Double glove.
16c. •Dispose of ethidium bromide stained items as hazardous waste.

17. Add gel running dye (1/5 volume) to each sample before loading them into the wells of the gel.
18. Run the gel at 80-120 volts (1-3 hours).
19. Visualize the gel under UV illumination (320 nm).

19a. •Wear a face shield when looking at UV light.
20. Store the gel in 1X TBE between uses.
20a. The gel can be used 1-20 times depending on how it is handled.
21. Satt 309: The higher band around 200 bps scores as the susceptible Essex allele. The lower band scores as the resistant Forrest allele. Note: there are other resistance and susceptibility alleles of varying sizes.
22. Satt 38: The higher band around 200 bps scores as the susceptible Essex allele. The lower band scores as the resistant Forrest allele.

Results and Discussion

Seed DNA extraction results in PCR products in about 62% of samples but ranges from 20-95% between batches of DNA preparations. The germination rate for cut seed in the greenhouse is not significantly affected. Field studies will commence in 1998. Leaf DNA extraction results in PCR products in about 95% of samples but ranges from 80-100% between batches of DNA preparations.

Segregation ratios for linkage group A2 and linkage group G are usually as expected. However, within some cross populations the segregation of linkage group G is distorted. In Manokin x Flyer F5 selected F6 lines on;y 5% of lines carried the Manokin allele on linkage group G, of these lines only 1% was SCN reistant. The marker Blt 65 (linked to SDS resistance) had an even distribution within the population, suggesting that it was not highly linked to any visible phenotypic variation of the selected parent plants. The distribution of SATT309 and SATT38 suggests that they were linked to a deleterious phenotype (by the breeders assessment) within the selected parent population. To create a soybean cultivar, this linkage needs to be broken by selective breeding between plants like 1056 (figure 5) that contain SCN resistance and 1050 (figure 5) that contain SDS resistance.

In Hartwig x Flyer segregation distortion favoured non-assortment of the alleles on linkage group G and A2 by about 10%. The selection against assortment was not strong and would not be evident in small populations. However, MAS on populations of this sort will provide a higher degree of selection than predicted theoretically.

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Correlation of Emergence and Canopy Closure to Yield in Group IV Soybean Lines

Introduction

High yield of soybean (*Glycine max* (L.) Merr.) is dependent upon several factors including seed emergence and canopy closure. Previous research relating emergence, or seedling vigor to yield have been inconsistent. Several studies have determined that a decrease in seedling vigor corresponded to lower yield (Johnson and Wax, 1978; Egli and TeKrony, 1979). Additional research has found no correlation between seedling vigor and final yield (Edje and Burris, 1971; Egli and TeKrony, 1979). Poor seedling emergence creates a situation for farmers where a decision must be made to replant (Willmot et al., 1989). Because of economic factors involved in replanting, it is of interest to determine if fast emerging lines have a yield advantage over slower emerging lines.

Seed yield has also been shown to be dependant upon light interception by the canopy (Ashley and Boerma, 1989) and additional canopy factors (Wells, 1991). Positive correlations between canopy photosynthesis and seed yield have been significant (Wells et al., 1982; Boerma and Ashley, 1988). In northern latitudes, plant spacings which minimize time to canopy closure were found to have maximum yield (Costa et al., 1980). It is therefore of interest to identify lines, which are able to rapidly close the canopy between rows.

Data has not been reported over the entire growing season relating emergence, canopy closure, and yield potential. The objective of this research was to determine if a correlation existed between yield potential and rapid emergence, and/or fast canopy row closure.

Materials and Methods

Cultivar Thorne (McBlain et al., 1993) was crossed to cultivar Spry (Bernard and Nickell, 1992). Approximately 110 individual F_2 plants were tagged and harvested separately in 1995. F_3 plants were grown in 1996 as plant row yield trials (96PRYT) with Resnik (McBlain et al., 1990) as a common border to minimize border row environmental effect. In 1997 the 28 highest yielding 96PRYT, Thorne, and Spry were grown as 4 row plots, 2 replications, at 2 locations of the Crop Science Research and Education Center (Cruse, IL and East Grein, IL). Plots were 4.5 m long with 76 cm row spacing. The Cruse location was planted on 4/29/97 and the East Grein location on 5/12/97. Visual emergence ratings were given for each plot on two different dates early in the growing season. A rating of 0 indicated no cotyledons were evident in the four rows of the plot, while a rating of 5 indicated approximately 90% of the four rows had cotyledons emerging from the soil level. Canopy closure dates were determined as the date when approximately 90% of the leaves in the 4 row plot were touching, enough to shade between the row. Maturity and lodging data were collected and the center 2 rows for each plot were harvested for yield. Seed moisture and seed quality data were recorded. Data were subject to ANOVA to determine significance of emergence scores, canopy closure date, maturity, lodging, moisture, and seed quality upon yield potential. Correlations were determined based on the means of the two reps within each location, and combining mean data at both locations.

Results and Discussion

The visual ratings for emergence appeared to have a high level of environmental influence, especially at the East Grein location. Several of these entries had large differences in the date of emergence when both replications are compared. This may be due to differences in soil temperature or other microenvironmental influences. In examining the correlation data at East Grein, emergence had a slight negative correlation to seed quality (Table 1). This would suggest that later emerging lines have lower seed quality. Emergence was not significantly correlated to canopy closure date, maturity, height, lodging, or yield. At the Cruse location, emergence had a slight correlation to maturity. This would suggest that later emerging lines also have later maturity (Table 1). Emergence was not correlated to canopy closure date, height, lodging, seed quality, or yield. When the means from both locations are pooled, the data suggests that emergence is not significantly correlated to any of the yield components (Table 1).

Scores for canopy closure date were consistent between replications at a location. However, lines at East Grein had canopy closure dates that were almost ten days earlier than at Cruse. This effect may be due to less lodging and more erect rows at the Cruse location. The data at the East Grein location reveals that canopy closure date is negatively correlated to lodging and yield (Table 1). This would imply that lines which have earlier canopy closure have more lodging and higher yields. Canopy closure date was not correlated to height, maturity, or seed quality. At the Cruse location, canopy closure date was negatively correlated to lodging (Table 1). The data suggest no correlation between canopy closure date and maturity, height, seed quality, or yield. Combining the means of both locations revealed that canopy closure date was negatively correlated to lodging (Table 1).

In general, the data presented would suggest that the relative rate of emergence does not have an effect on later developmental traits, including yield. Lines from this population appear to be able to compensate for slower emergence in cool soil by taking advantage of warmer growth conditions later in the season. The canopy closure data had a negative correlation to lodging, but was not highly correlated to other yield components. Canopy closure date may therefore be useful in a breeding program as an indirect selection against lodging.

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Table 1. Correlations (r) comparing trait means of a Spry x Thorne F_3 population.

	Trait					
Trait	canopy closure†	maturity‡	height§	lodging#	Seed quality¶	yield††
emergence	0.03	-0.31	-0.24	0.17	-0.14	-0.13
canopy closure	-	-0.17	-0.05	-0.77	-0.03	-0.22
Location = Cruse, IL						
emergence	0.11	-0.03	-0.12	0.16	-0.23	-0.12
canopy closure	-	-0.30	-0.14	-0.67	-0.19	-0.44
Location = East Grein, IL						
emergence	0.07	-0.21	-0.16	0.17	-0.10	-0.13
canopy closure	-	-0.29	-0.10	-0.72	-0.14	-0.37
Location = Cruse, IL + East Grein, IL						

† Date when approximately 90% of the 4 row plot had leaves touching.
‡ Date when 90% of all pods are physiologically mature.
§ Height (inches) from ground to highest node.
# Visual rating of lodging (0 = all plants erect, 5 = no plants erect).
¶ Visual rating of quality (0 = perfect seed, 5 = extremely poor seed).
†† Dry weight seed yield, all plot measurements adjusted to 13% moisture standard.

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Establishment of a Cytogenetic Map of Soybean: Current Status

The cytogenetics of the cultivated soybean [*Glycine max* (L.) Merr., 2n = 2x = 40] has lagged far behind that of other major crops (Palmer and Kilen, 1987; Singh and Hymowitz, 199 1). In the soybean, approximately 250 morphological and isozyme markers are available. To date, 19 classical genetic linkage groups consist of 63 classical markers and a few linkage groups are constructed by only two loosely associated marker genes (Palmer and Hedges, 1993). Recent advances in the soybean molecular mapping have resulted in the establishment of several molecular maps, which included more than 1000 RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeat) and AFLP (Amplified Fragment Length Polymorphism) markers (Akkaya et al., 1995; Keim et al., 1997; Lark et al., 1993; Rafalski and Tingey, 1993; Shoemaker and Olson, 1993). However, most of the known genes in the soybean have not been located on chromosomes. Nearly none of all the genetic linkage groups and molecular maps have been associated with chromosomes.

Primary trisomics are useful for locating genes on the chromosomes and for associating a linkage group with a particular chromosome in several economically important diploid plant species. Since Singh and Hymowitz (1988) established the first cytological map in soybean based on pachytene chromosome analysis, we have initiated a project to isolate and identify primary trisomics (2n=41) in this important crop (Singh and Hymowitz, 1991; Ahmad et al., 1992; Ahmad and Hymowitz, 1994; Xu et al., 1997). This report further updates progress and current status of our study.

The source of the primary trisomics was the progenies of the 38 aneuploid lines with 2n 41 to 43 chromosomes (Table 1). These lines were initially isolated from several meiotic mutants (asynaptic and desynaptic) and male sterile lines. The pedigrees of 34 lines were known, but four lines were of unknown origin. Majority of the aneuploid lines were provided by Dr. R. G. Palmer, USDA, ARS, Iowa State University, Ames, Iowa. Lines T1 90-39, T1 90-47-1, and T1 90-47-3 were generated in the Soybean Cytogenetics Laboratory at Urbana-Champaign.

The cytological methods for identifying the primary trisomics were essentially the same as reported previously (Singh and Hymowitz, 1991; Ahmad et al., 1992; Ahmad and Hymowitz, 1994). The mitotic study was conducted based on a modified staining procedure for mitotic chromosomes (Xu et al., 1998). The meiotic studies, particularly pachytene chromosome analysis, were conducted according to Singh and Hymowitz (1988). Primary trisomics were identified based on meiotic pairing and chromosome morphology (i.e., chromosome length, centromere position, and distribution of euchromatin and heterochromatin) of the trivalents at pachynema stage. Since primary trisomics were of diverse genetic backgrounds, they are being backcrossed into a uniform genetic background by using the soybean cultivar 'Clark 63' as a recurrent parent. The tetrasomics were identified from the selfed progenies of the primary trisomics based on somatic chromosome count, morphological features, and meiotic chromosome pairing.

Currently, we have isolated and identified all possible 20 primary trisomics and related tetrasomics. They were tentatively designated in descending order as Triplo 1 (carrying the longest chromosome) to Triplo 20 (carrying the shortest chromosome) and Tetra I to Tetra 20. In this report, an appropriate rearrangement in triplo number was made based on the new cytological evidence. The updated 20 primary trisomics and related tetrasomics are listed in Table 2 and Table 3, respectively. Some primary trisomics and tetrasomics exhibit diagnostic morphological features, such as big pods and big seeds in Triplo 1, revolute leaflets in Triplo 8, small seeds in Triplo 13, shrunken seeds in Triplo 11, pseudo-glaborus leaves and big roots in Tetra 3, and compressed pods in Tetra 7.

These primary trisomics and tetrasomics are being tested for locating various markers to a particular chromosome in our laboratory. This study will eventually associate conventional genetic linkage groups and molecular maps with the cytogenetic maps as have been done for maize, barley, rice, and tomato.

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Table 1. List of aneuploid lines as source of primary trisomics in soybean.

Aneuploid lines	2*n*	Origin
Tri A	41	Asynaptic mutant T241†
Tri C	41	Desynaptic mutant T258‡
Tri D	41	Same origin as Tri C
Tri S	41	Neutron-irradiated 'Hodgson' §
T 170	41	Tissue culture-induced 'funman' sterile mutant ¶
T177	41	Same origin as T170
FA 1-2	41	Hybrids of a *ms1ms1* plant (2n=42) by 'Wye' #
FA 1-4	41	Same origin as FA 1-2
FA 1-7	41	Same origin as FA 1-2
FA 1-8	41	Same origin as FA 1-2
A88A-21	41	T286 (*ms1ms1*)††
A88B-13	41	Same origin as A88A-21
SRF-70:11	41	Progeny of asynaptic mutant found in the F_5 of SRF 150P × Northrup King‡‡
SRF-70:27	41	Same origin as SRF-70:11
SRF-70:28	41	Same origin as SRF-70:11
SRF-70:120	41	Same origin as SRF-70:11
SRF-70:121	41	Same origin as SRF-70:11
SRF-70:TH216	41	Same origin as SRF-70:11
KS:TH745	41	Progeny of asynaptic mutant found in the F_3 of T210 df 2 × KS35-1-2 yellow dwarf‡‡
KS:TH747	41	Same origin as KS:TH745
KS:TH752	41	Same origin as KS:TH745
KS:TH764	41	Same origin as KS:TH745
KS:TH772	41	Same origin as KS:TH745
KS:TH775	41	Same origin as KS:TH745
MD70	41	Progeny of asynaptic mutant found in the F_4 of PI 154402 × PI 86062‡‡
MD76	41	Same origin as MD70
T190-39	41	Hybrids of 'Funman' sterile by 'Clark 63'
T190-47-1	41	Same origin as T190-39
T190-47-3	41	Same origin as T190-39
A84C1-5-4	43	
A84C1-5-4-8-1	41	Hybrid of A84C1-5-4 by 'Clark 63'
A84C1-5-8	42	
A84C1-5-8-3	41	Derived from A84C1-5-8
A84C1-5-8-5	41	Derived from A84C1-5-8
A84C1-5-10	42	
A84C1-5-10-15	41	Derived from A84C1-5-10
A84C1-5-11	43	
A84C1-5-11-18	41	Derived from A84C1-5-11

†Palmer, 1974; ‡Palmer and Heer, 1976; §Sadanaga and Grindeland, 1984; ¶Graybosch et al., 1987; #Zhang and Palmer, 1990; ††R. G. Palmer, personal communication; ‡‡H. Skorupska, personal communication.

Table 2. Triplo number, code, recurrent generation with 'Clark 63' and source of primary trisomics (2n=41) in soybean.

Triplo	Code	Generation	Source (2*n*)
1	UT95-102	BC_4	Tri C (41)
2	UT95-103	BC_4	KS:TH747 (41)
3	UT95-106	BC_4	KS:TH775 (41)
4	UT95-108	BC_4	Tri D (41)
5	UT95-109	BC_4	Tri A (41)
6	UT95-120	BC_4	SRF70:11 (41)
7	UT95-20	BC_2	A84-C1-5-4 (43)
8	UT94-18	BC_4	A84-C1-5-10 (42)
9	UT97-54	BC_1	MD76 (41)
10	UT95-142	BC_2	A88B-13 (41)
11	UT94-33	BC_4	T170 (41)
12	UT96-57	BC_4	SRF70:TH216 (41)
13	UT95-135	BC_4	Tri S (41)
14	UT95-125	BC_4	MD70 (41)
15	UT94-147	BC_4	SRF70:120 (41)
16	UT95-10	BC_2	T190-47-1 (41)
17	UT94-8	BC_4	A84-C1-5-11 (43)
18	UT96-63	BC_2	KS:TH745 (41)
19	UT94-39	BC_4	T177 (41
20	UT93-20	BC_4	A88A-21 (41)

Table 3. Tetrasomics (2n=42) derived from progenies of primary trisomics in soybean.

Tetra	Code	Source (2*n*)
1	UT94-103	Tri C (41)
2	UT95-156	KS:TH747 (41)
3	UT94-167	KS:TH775 (41)
4	T92-4-1	Tri D (41)
5	T92-5-9	Tri A (41)
6	UT95-175	SRF70:11 (41)
7	UT96-82	A84-C1-5-4 (43)
8	UT95-158	A84-C1-5-10 (42)
9	UT97-59	MD76 (41)
10	UT93-11	A88B-13 (41)
11	UT94-150	T170 (41)
12	T92-10-6	SRF70:TH216 (41)
13	UT96-131	Tri S (41)
14	T91-4	MD70 (41)
15	T92-19-3	SRF70:120 (41)
16	UT96-135	T190-47-1 (41)
17	UT95-165	A84-C1-5-11 (43)
18	UT96-81	KS:TH745 (41)
19	UT95-166	T177 (41)
20	UT96-128	A88A-21 (41)

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Genetic Analysis of Phytophthora Rot Resistance in the Soybean PI 567.496

Introduction

Plant Introduction (PI) 567.496 is resistant to hypocotyl inoculation (Moots et al., 1983; Schmitthenner and Bhat, 1994) of races 1, 3, 4, 5, 7, 13, and 25 and susceptible to races 12, 17, and 20 of *Phytophthora sojae* (Table 1). Currently, there is no identified gene with a similar response pattern, although a gene combination of *Rps1b* (Mueller et al., 1978) with *Rps3a* (Mueller et al., 1978) or *Rps5* (Buzzell and Anderson, 1981) would give this response pattern(Table 1). The objective of this study was to determine the number of genes controlling the resistance found in PI 567.496.

Materials and Methods

In the summer of 1994, a cross (LNX94170) was made between 'Williams' (Bernard and Lindahl, 1972) X PI 567.496 at the Crop Sciences Research and Education Center in Urbana, IL. One hundred F_2 seeds from the cross were saved in cold storage and the remaining F_2 seeds were planted in the field in 1996. Single F_2 plants were tagged and harvested from the F_2 population to form the F_2 derived F_3 families ($F_{2:3}$ families).

During the winter of 1996-1997, the saved F_2 seeds and the seeds of 70 $F_{2:3}$ families were planted in the greenhouse for evaluation of their response to race 1 of *P. sojae*. Plants were inoculated by the hypocotyl inoculation method with zoospores (Moots et al., 1983). Parental lines were included in the experiment to check the virulence of the pathogen. Five days after inoculation the number of dead and living plants for the F_2 population and each $F_{2:3}$ family was recorded. When screening with race 1, a 15 resistant to 1 susceptible ratio would be expected if two genes were segregating for resistance to race 1, which would be the case if a combination of *Rps1b* with *Rps3a* or *Rps5* were present. A ratio of 3 resistant to 1 susceptible would be expected if one gene were segregating for resistance to race 1. The data were analyzed by the chi- square test for goodness of fit to expected ratios.

Results and Discussion

The F_2 population from the cross LNX94170, inoculated with *P. sojae* (race 1), segregated in a ratio of approximately 3 resistant to 1 susceptible. The calculated chi-square probability for the F_2 population fitting a ratio of 3 resistant to 1 susceptible was 0.047 (Table 2). The poor chi-square fit of a 3 to 1 ratio is due to an excess of only 8 plants in the susceptible class. $F_{2:3}$ families were classified as resistant, heterogeneous, or susceptible. The chi-square test for a goodness of fit of a 1 resistant to 2 heterogeneous to 1 susceptible ratio for $F_{2:3}$ families indicated a good fit with a chi-square probability of 0.73 (Table 2). Heterogeneous and resistant families were combined, and a chi-square probability of 0.49 indicated a good fit to a 3 resistant or heterogeneous family to 1 susceptible family ratio (Table 2). A chi-square test was used to test the fit of the F_3 plants to a 5 resistant to 3 susceptible ratio. The chi-square probability was 0.0003, indicating a poor fit (Table 2). Resistant F_2 and F_3 plants were believed to have been misclassified as susceptible due to a high concentration of zoospores used for inoculating and the aggressiveness of this culture of race 1.

The data from the $F_{2:3}$ families suggests a single gene controlling resistance to race 1. In conclusion, the resistance in PI 567.496 to race 1 is controlled by a single gene and not conditioned by a combination of previously identified genes. However, this does not eliminate the possibility of a new gene in combination with a previously identified gene.

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Table 1. Reaction of parents, and *Rps1b*, *Rps3a*, and *Rps5* to hypocotyl inoculation of ten races of *Phytophthora sojae*.

Parent or	Race Reaction									
Gene	1	3	4	5	7	12	13	17	20	25
Williams	S†	S	S	S	S	S	S	S	S	S
PI 567496	R	R	R	R	R	S	R	S	S	R
Rps1b	R	R	R	R	R	S	R	S	S	S
Rps3a	R	R	R	R	S	S	R	S	S	R
Rps 5	R	R	S	R	S	S	R	S	S	R

†S=susceptible, R=resistant

Table 2. Reaction of F_2 plants, $F_{2:3}$ families, and F_3 plants from the cross, Williams x PI 567.496 to inoculation with Race 1 of *Phytophthora sojae*.

Generation	Res.†	Het.‡	Susc.§	Theoretical Ratio	Chi-Square Probability.
	no. plants				
F_2	65		33	3:1	0.0474
$F_{2:3}$ families	18	32	20	1:2:1	0.7303
$F_{2:3}$ families#	50	20		3:1	0.4901
F_3	2462		1658	5:3	0.0003
Williams	0		30		
PI 567496	27		5		

† Observed resistant plants and families; ‡ Observed heterogeneous families; § Observed susceptible plants and families; # Resistant and heterogeneous families combined.

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Genetic Analysis of Tolerance to *Phytophthora sojae* in the Soybean Cultivar, Jack

Introduction

Extensive use of race-specific resistance in soybean [*Glycine max* (L.) Merr.] for controlling Phytophthora rot, caused by *Phytophthora sojae* (Faris and Sabo, 1989), has promoted the selection for other races which are virulent to commonly deployed resistance genes. Alternative control strategies have been examined (Schmitthenner and VanDoren, 1985) and are being implemented by soybean breeders to maintain control of this disease. The use of tolerant or field resistant cultivars may be less selective for virulent races and may provide a sustained level of control (Schafer, 1971; Schmitthenner, 1985; Thomison et al., 1988).

The cultivar, Jack (Nickell et al., 1990), is not known to carry genes for race-specific resistance to *Phytophthora* but does perform well in field disease conditions. When Jack is compared to the cultivar, Macon (Nickell et al., 1996), which is not known to carry genes for race-specific resistance, Jack displays an intermediate response to hypocotyl inoculation (Moots et al., 1983) of *P. sojae*. Macon is uniformly susceptible to hypocotyl inoculation. The objectives of this research were to study the inheritance of tolerance in Jack and ascertain the potential of selecting progeny with high levels of tolerance.

Materials and Methods

The study evaluated progeny from the cross, LNX94018 of Jack X Macon. One hundred F_5 lines derived from an F_4 population ($F_{4:5}$ lines) were evaluated. A modified inoculum layer method, similar to the method of Schmitthenner and Bhat (1994), was used for evaluation. Lines grown with and without Race 1 of *P. sojae* were compared for the number of live plants and for the average plant height at 28 days after planting. The percent performance of each line was calculated with the following equation: percent performance = [(no. live plants in inoculated treatment * height)/(no. live plants in uninoculated treatment * × height)] * 100.

Results and Discussion

The mean tolerance scores of the lines were ranked and a means comparison test of the lines and each parent was used to separate differences in the lines and each parent. Large differences between the mean ranks of lines and the mean ranks of the parents were needed to separate the lines and the parents. The mean rank of Jack was significantly higher than the mean rank of Macon. When Jack was compared to each of the lines, sixty-six lines were significantly lower ($P>0.05$) than Jack and thirty four lines were not significantly different from Jack. When Macon was compared to each of the lines, nine lines were significantly higher than Macon, sixty-eight lines were not significantly different from Macon, and twenty-three lines were significantly lower than Macon. From the results of the means separation test for each parent, lines could be separated from both parents, one parent, or neither parent. There were nine of the lines that were not different from Jack and were significantly higher than Macon. Twenty-five lines were unable to be classified different from either parent. Figure 1 shows the distribution of the lines and parents by their respective mean tolerance score. The continuous variation that occurred in tolerance scores of lines would suggest that tolerance to *P. sojae* is controlled by more than one gene, which agrees with results of previous tolerance studies (Buzzell and Anderson, 1982; Walker and Schmitthenner, 1984). Epistatic gene effects may explain the high number of lines with lower levels of tolerance, and the low number of $F_{4:5}$ lines with tolerance levels like Jack.

An estimate of broad sense heritability for tolerance was calculated by the components of variance method. The heritability estimate for tolerance was 59.6% on an entry mean basis. This estimate of heritability for tolerance is lower than those previously reported by Walker and Schmitthenner (1984), and Buzzell and Anderson (1982). An estimate of heritability of 59.6% would indicate that selection for tolerant lines in early generations would be effective and genetic gain would be expected. Jack appears to be a suitable donor parent for *Phytophthora* tolerance and has the potential to provide high seed yield and soybean cyst nematode resistance as well. Breeding for tolerant varieties may offer a sustainable control of Phytophthora rot in the future.

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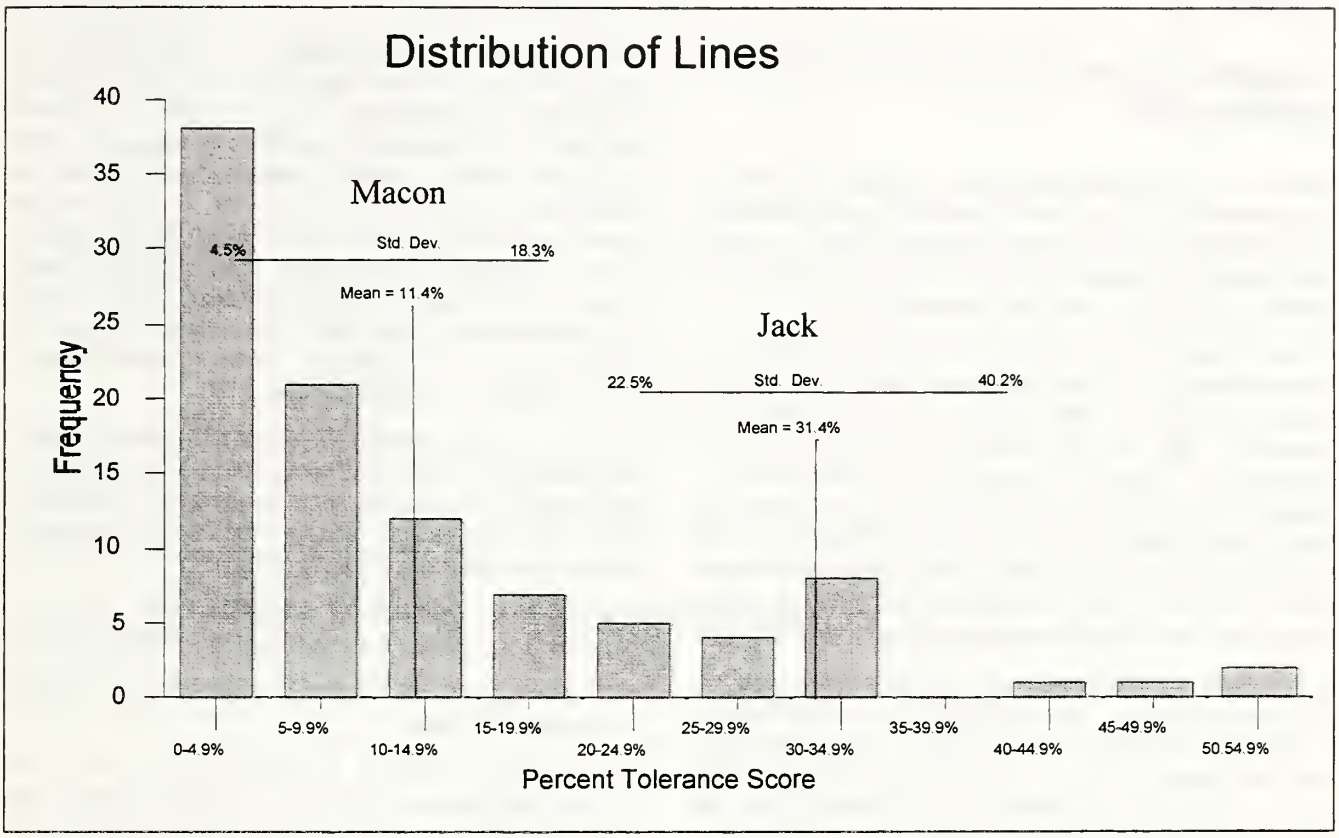
Figure 1. Distribution of $F_{4:5}$ lines with respect to tolerance scores, and the mean and standard deviations for each parent.



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Management of the USDA Wild Perennial Glycine Collection, 1997

Under a specific cooperative agreement between the University of Illinois and the USDA, the wild perennial Glycine collection is being maintained at the University of Illinois. Table 1 contains the names of the species, somatic chromosome numbers, and number of accessions. The major points are as follows:

1. Four shipments containing a total of 76 new accessions were received during 1997. Two of these originated in Australia, and include material from a 1996 collecting trip by Grace, Brown and Hymowitz. Two were from Taiwan (see note about Glycine collection trip to Taiwan). One of these was a shipment of a possible new species, *G. dolichocarpa*. The other contained seven new accessions that were collected this year by Dr. Hymowitz during a trip to Taiwan. One of these accessions is used in traditional Chinese medicine as an herbal tea; we hope to multiply this line and have it tested for pharmacological activity.

2. A number of accessions are difficult to grow through maturity and seed production. To counter this, these lines have been intensively propagated by means of cuttings. Seeds are surface sterilized and germinated in autoclaved media. This has allowed some accessions of *G. tomentella* to be multiplied for the first time. Some plants are reluctant to set pods - hand pollination of flowers with a toothpick increases pod set in some accessions of *G. canescens, G. falcata* and G. *falcata*.

3. In order to protect employees from dermal exposure to toxins, and in response to the development of multiple pesticide resistance in greenhouse pests, the perennial greenhouse is on a biological control regime. The following measures have been adopted:

a. Screening: all vents have been screened to prevent an influx of nuisance and pest insects
b. Appropriate chemicals: insecticidal soaps are useful for spot treatments as they have no residual toxicity and no harmful fumes.
c. Predaceous and parasitic organisms:
    1. Mealybugs: *Leptomastix* wasps (parasites).
    2. Whiteflies: Encarsia (parasitic wasp).
    3. Thrips: Orius predatory bugs, and *Neoseilulus cucumeris* predaceous mites.
    4. Spider mites: *Mesoselius longipes*, a species of predaceous mites that tolerates low humidity. The plants are also hosed down to physically disrupt spider mite colonies.
    5. Fungus gnats: Gnatrol, a *Bacillus thuringensis* drench.
    6. Aphids: *Aphidius colemani* parasitic wasps.

These organisms may be obtained from any of a number of companies, including the following:

IPM Laboratories, Inc.
P. O. Box 300
Locke, NY 13092-0300

Biotactics
7765 Lakeside Drive
Riverside, CA 2509

Results have been quite encouraging. The high initial costs of the control organisms have been offset by the resistance of the greenhouse to reinfestation and a reduction in chemical expenses. With increasing pesticide regulation and longer reentry restrictions, reducing pesticide levels makes the greenhouse more useful to researchers. As no biological controls exist for fungus diseases, the plants are drenched with Banrot, a broad-spectrum fungicide, about every four weeks. They are also sprayed with Rubigan to prevent powdery mildew. The beneficial insects seem able to tolerate these fungicides. As required by law, Jean Burridge is a licensed Public Applicator, Demonstration and Research category.

4. During the year, 28 seed requests for wild perennial material were received. A total of 510 packets of seed was shipped. A standard packet contains 5 seeds of an accession. Domestically, seed was shipped to Alabama, Illinois, and Wisconsin. Internationally, seed was shipped to Taiwan.

5. Voucher specimens of all accessions grown out in the greenhouse were placed in the Crop Evolution Herbarium (CEL).

6. The inventory of the collection is maintained on a Gateway 2000 personal computer.

7. Twenty-eight packets of 50 seed each were sent to the National Seed Storage Laboratory in Fort Collins, Colorado for long term storage. Thus far, 806 accessions have been sent to this facility. Another set, containing 10 seeds per packets, was sent to Dr. R. L. Nelson, Curator, USDA Soybean Germplasm Collection, Urbana, IL. PI numbers are requested when an accession has been successfully multiplied. Seeds of all accessions in the collection are stored in envelopes, in a milk cooler set to 4 degrees Fahrenheit.

8. To request seed, please write, e-mail, FAX, call or visit our web site:

a. Dr. T. Hymowitz
    Department of Crop Science
    University of Illinois
    AE-110 Turner Hall
    1102 S. Goodwin Ave.
    Urbana, IL 61801
    USA
b. soyui@uiuc.edu
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d. TEL: (217) 133-9454
e. WWW: http://biometry .agn.uiuc.edu/~hymowitz/

Table 1. Wild perennial *Glycine* species, somatic chromosome number, and number of accessions.

	Species	2n	Number of Accessions
1.	*G. albicans*	40	2*
2.	*G. arenaria*	40	5
3.	*G. argyrea*	40	13
4.	*G. canescens*	40	80
5.	*G. clandestine*	40	138
6.	*G. curvata*	40	9
7.	*G. cyrtoloba*	40	49
8.	*G. falcata*	40	14
9.	*G. hirticaulis*	40	1*
		80	1*
10.	*G. lactovirens*	40	2*
11.	*G.* latifolia	40	46
12.	*G. latrobeana*	40	12*
13.	*G. microphylla*	40	32
14.	*G. pindanica*	40	5
15.	*G. tabacina*	40	14
		80	131
		?	91
16.	*G.tomentella*	38	22
		40	58
		78	55
		80	53
		?	132
			1036

* Recalcitrant species with regard to seed multiplication

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Plant Exploration Trip to Taiwan and the Islet of Kinmen (Quemoy)

From September 1 to September 11, 1997, the National Science Council, Taiwan and the Center for East Asian and Pacific studies, University of Illinois sponsored a plant exploration trip to the Taitung District of Taiwan and to the islet of Kinmen, to collect wild perennial relatives of the soybean. My hosts and collaborators for my visit to Taiwan were Professor J. S. Hsieh, Department of Agronomy, Taiwan National University and Dr. Caroline Hsing, Botanical Institute, Academia Sinica.

In the Tonko and Gialuland areas of the Taitung District, G. dolichocarpa Tateishi and Ohashi was located. Subsequent studies at Illinois suggest the plants are members of the G. tomentella 80 chromosome allopolyploid species complex.

On Kinman, seeds of G. tomentella and G. tabacina were collected from sandy sites.

To my surprise I found that on Kinman, The Wu-Chi Co., was selling herbal tea bags and herbal tea capsules produced from the roots of G. tomentella. This is the first known ethnobotanical use of a wild perennial relative of the soybean. I visited several fields growing G. tomentella and interviewed the company managers. It was obvious that soybean rust problems need to be resolved if the crop is to survive as a commercial operation on Kinman.

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Segregation Analysis of Light Pink Flower x White Flower F_2 Soybean Populations

Introduction

The epistatic interaction of several loci affects flower color in soybean (*Glycine max* (L.) Merr.). Purple flowers arise due to the production of anthocyanin in floral tissues, controlled by dominant *W1* and *W4* loci (Groose et al., 1988). Homozygous *w1w1* flowers are lacking in anthocyanin production and are white. Genotypes *W1__ W3W3 w4w4* were reported to have purple pigmentation confined to the throat of the flower petal (Hartwig and Hinson, 1962). The *w3* allele diffuses coloration to create near-white flowers in genotypes *W1 __ w3w3 w4w4*. In the presence of *W1, wmwm* generates magenta flowers (Buzzell et al., 1977) and *wpwp* conditions pink flowers (Stephens and Nickell, 1991).

Light pink flower phenotypes were observed in the field from populations derived from the cross LN89-5322-2 (pink flower) x P.I. 423.948A (light purple flower). It was of interest to determine if the light pink phenotype was a result of influence of *w3, w4,* or a novel locus. The objective of this study was to evaluate three F_2 populations with light pink flower phenotype to determine segregation at the *W1, W3, W4*, and *Wp* loci.

Materials and Methods

Light pink flowered line LN95-16312 was crossed to white flower cultivars Jack (Nickell et al., 1990) and Saline (Owen et al., 1994), while line LN95-16308 (light pink flower) was crossed to Saline. The light pink genotype is assumed to be *W1W1W3W3w4w4wpwp*, and the genotypes of white flower cultivars is *w1w1w3w3W4W4WpWp*. F_2 populations from these crosses were grown in 1997 at the Crop Science Research and Education Center, Urbana IL. The populations were planted as two row plots, 4.5 m long, with 76 cm spacing between rows. Flower colors of each plant were recorded and approximately 10 plants from each phenotype observed were tagged. A model describing 4 segregating flower color genes and the expected frequencies was determined. Flower color data observed in the field was subject to chi-square analysis to compare goodness-of-fit with expected ratios.

Results and Discussion

Several different flower color phenotypes were observed in the F_2 populations. The purple flower phenotypes were classified as dark purple if the pigmentation was a dark indigo blue/purple, while normal purple flowers were a crimson red/purple color. The shades of pink flowered phenotypes were difficult to separate as sunlight fades new flowers relatively quickly. In addition, for this study near-white or purple-throat phenotypes were not separated from within the white classified flowers due to time limitations.

In comparing population 95266 with the model for four independent segregating genes, the frequency of total purple plants was as expected (Table 1). However, the population had more normal purple phenotypes and less dark purple phenotypes than would be expected. In addition, more pink phenotypes and less white phenotypes were observed. This error may have resulted due to missclassifying white phenotypes as light pink. When the pink frequency and white frequency are added, the population is not significantly different from the expected model.

Population 95267 had a slightly higher frequency of purple phenotypes than would be expected, but not significantly different than the model (Table 1). This population also had a higher pink frequency and a lower white frequency when compared to the model. The error may be attributed to misclassification of white phenotypes as light pink.

Population 95268 had a higher frequency of purple and pink phenotypes than would be expected, and a lower frequency of white phenotypes (Table 1). The deviation from the expected model for this population may be due to the smaller size, and misclassification of white phenotypes.

The data from populations 95266 and 95267 fit the expected 3:1 ratio for the *W1, W4*, and *Wp* loci at the 0.05 significance level (Table 2). Data for the *W3* locus did not fit an expected 3:1 ratio, but rather fit a 1:1 ratio at the 0.05 significance level for both populations (Table 2). It is possible that some phenotypes were missclassifed for *W3*. The *w3* allele acts genetically to diffuse coloration in flower petals, but sunlight will act environmentally to fade flower color as the day progresses. Therefore, it is possible that some phenotypes were mistakenly classified as light purple or light pink instead of the normal purple or normal pink. It is also possible that the light pink parental plant was heterozygous for *W3*. A heterozygous plant would produce approximately half dominant and half recessive F2 progeny. A 1:1 ratio was observed for populations 95266 and 95367 at the *W3* locus (Table 2).

The data from population 95268 fit the expected 3:1 ratio for the *W4* and *Wp* loci at a significance level of 0.05 (Table 2). The *W1* and *W3* data do not fit the 3:1 ratio that would be expected for single gene Mendelian segregation. The sample size for this population was small in that it consisted of only 2 rows and therefore may not accurately represent the entire population. For the *W1* locus, it is possible that some of the white flowered lines were missclassified as light pink, however this seems unlikely since the white flower had a yellowish tint at the base of the petals, which was lacking in light pink phenotypes. The *W3* data for this population fits a 1:1 ratio, suggesting possible missclassification, or that the light pink parent was heterozygous at the *W3* locus (Table 2).

The data from the three populations suggests that light pink flowered line LN95-16312 may arise due to the epistatic interaction upon *wp* by *w3* and/or *w4*. The original light pink plant

appears to be heterozygous at the *W3* locus, as all F_2 populations fit a 1:1 ratio instead of the expected 3:1 (Table 2). The data does not exclude the possibility of other novel loci interacting to generate the light pink phenotype, which will need to be confirmed in future linkage analyses.

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Table 1. Expected and observed frequencies for light pink flower x white flower F_2 populations.

Cross: *w1w1w3w3W4W4WpWp* (white) x *W1W1W3W3w4w4wpwp* (light pink)

Flower Phenotype	Expected Frequencies†	Observed Frequencies Population‡ 95266	95267	95268
Dark Purple	0.316	0.170	0.141	0.159
Normal Purple	0.106	0.256	0.306	0.275
Light Purple	0.106	0.098	0.104	0.135
Total Purple	0.527	0.524	0.551	0.570
Dark Pink	0.035	0.002	0.006	0.000
Normal Pink	0.106	0.145	0.168	0.193
Light Pink	0.035	0.096	0.062	0.053
Total Pink	0.176	0.243	0.236	0.246
Near White	0.035			
Normal White	0.262	0.233	0.213	0.183
Total White	0.297	0.233	0.213	0.183

† Frequencies expected for four independent heterozygous genes; *W1, W3, W4* and *Wp*.
‡ Pedigrees: 95266 = Jack x LN95-16312; 95267 = Saline x LN95-16308; 95268 = Saline x LN95-16312.

Table 2. Chi-square analysis of F_2 populations segregating for flower color loci.

	Flower loci											
	W1/w1†			*W3/w3*‡			*W4/w4*†			*Wp/wp*†		
Popul.	Obs.	Exp.	P§	Obs.	Exp.	P	Obs.	Exp.	P	Obs.	Exp.	P
95266	375	366.8		264.5	244.5		341.5	348.8		370	366.8	
	114	122.3	0.389	224.5	244.5	0.071	123.5	116.3	0.437	119	122.3	0.734
95267	380	362.3		228.3	241.5		326.25	324		369	362.3	
	103	120.8	0.062	254.8	241.5	0.228	105.8	108	0.803	114	120.8	0.228
95268	169	155.3		102.8	105		131.8	135.8		156	155.3	
	41	51.8	0.067	107.3	105	0.756	49.3	45.3	0.492	51	51.8	0.904
All	924	886.5		595.5	591		799.5	808.5		895	884.3	
Rows	258	295.5	0.012	586.5	591	0.794	278.5	269.5	0.527	284	294.8	0.794

† Data shown fit for 3:1 expected model
‡ Data shown fit for 1:1 expected model
§ P= Probability that deviation is due to chance alone

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Allelism Tests of Mutants Generated by Instability at the *k2 Mdh1-n y20* Chromosomal Region

Three distinct genetic loci define the *k2 Mdh1-n y20* chromosomal region in soybean. The *k2* allele conditions the tan saddle seed coat phenotype, the *Mdh1-n* allele conditions mitochondrial male dehydrogenase 1 (*Mdh1)* null phenotype, and the *y20* allele conditions yellow foliage phenotype. The precise gene order is not known.

We introduced the *w4-m* and *Y18-m* mutable systems into the three independent sources of tan saddle seed coat mutants, T239 (*k2*), T261 (*k2 Mdh1-n*), and L67-3483 (*k2*). A total of 12 bright yellow mutants were isolated with tan saddle seed coat, malate dehydrogenase 1 null phenotypes. Among the 11 F2 mutant families, 10 of 381 were associated with T239 genetic background, and one out of 323 F2 mutant families was associated with T261 genetic background.

Our objectives were to characterize genetically the 12 new mutants by allelism test, and to determine the mutation frequency in a control population. The genetic mechanism responsible for generating this high frequency of instability is reported elsewhere (Chen and Palmer, accepted).

Materials and Methods

Soybean mutants and parents used in cross-pollinations are listed in Table1.

Soybean crossing techniques were adapted from Walker et al. (1979) to obtain cross-pollinations. Foliage, seed coat, flower, and pubescence colors were used as morphological markers to verify successful cross-pollinations. Crosses were made at the Bruner Farm near Ames, Iowa, in the summers of 1992-1994. F1 seeds were advanced to F2 seeds at the Iowa State University-University of Puerto Rico Soybean Nursery, at the Isabela Substation, Isabela, Puerto Rico. F2 seeds were planted at the Bruner Farm in the summers of 1993-1995. Seedling traits were classified two weeks after planting. Seed coat colors were evaluated at maturity. The segregation of foliage color was recorded in the F2 entries segregating new mutants. The bright yellow plants were harvested individually at maturity for further inheritance and allelism tests.

Crosses for allelism tests at the *k2* or *Mdh1-n* loci for the bright yellow mutants were made at the Bruner Farm and in growth chambers in the summer of 1995. Crosses for allelism tests at the *y20* locus for the bright yellow mutants were conducted in the USDA greenhouse at Iowa State University in the spring of 1995 and 1996. Seed coat colors were checked at maturity; and F1 and F2 seedling traits were evaluated in the sandbench in the USDA greenhouse at Iowa State University.

Starch gel electrophoresis was adapted from Cardy and Beversdorf (1984 a, b) to determine malate dehydrogenase [EC 1.1.1.37] isozyme banding patterns. Seeds were placed on germination paper at 37°C in a dark growth chamber. Electrophoretic samples were taken from the cotyledons of four-day-old seedlings by using a 100-ul micropipette. The samples were stored at -70°C until assayed. Starch gels were prepared by using 11.78% starch concentration with "B" gel and electrode buffer system (pH 6.5). Gels were run at a constant power of 9.5 W for 5.5 h in a 4°C chromatography cooling chamber.

Results and Discussion

Allelism tests at the *Mdh1-n* or the *k2* loci were conducted with these 12 independent tan saddle seed coat, malate dehydrogenase 1 null, and bright yellow mutants. T261 (*k2 Mdh1-n*) and T239 (*k2*) were used to test the *Mdh1* banding pattern in F1 hybrid seed and to evaluate the seed coat color in F2 plants (Table 2). All F1 seeds derived from crosses with T261 were *Mdh1* nulls, suggesting allelism at the *Mdh1* locus for the malate dehydrogenase 1 null (Table 2). All the F1 and F2 seeds derived from allelism tests were tan saddle, indicating allelism at the *k2* locus for the tan saddle seed coat (Table 2).

Allelism tests at the *y20* locus were made with the 12 bright yellow mutants by crossing with T325 (*Mdh1-n y20*) and by their intercrosses. All the F1 and F2 plants were yellow foliage, suggesting that all 12 bright yellow mutants were allelic at the *y20* locus (Table 3). Therefore, the 12 mutants with tan saddle seed coat, malate dehydrogenase 1 null, and bright yellow phenotypes were new mutants at the *k2 Mdh1-n y20* chromosomal region in soybean.

Eleven out of 12 new mutants at the *k2 Mdh1-n y20* chromosomal region were associated with T239 genetic background. To test whether the *k2* allele in T239 was responsible for generating these new mutants, Harosoy, the near-isogenic line of T239, was crossed with A1937, X1878, X2717, X2937, and Lincoln (parents of the *w4-m* and *Y18-m* mutable systems). Nine cross combinations (including reciprocal crosses) gave a total of 883 F2 families. No yellow mutants were observed in these F2 families (Table 4). Thus, we conclude that the *k2* allele in T239 was responsible for the bright yellow mutations described in this report.

References

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Table 1. Description of soybean lines used in crosses and new mutants at the *k2 Mdh1-n y20* chromosomal region

GT [a]	Strain no.	Gene symbol	Description
T239	L63-365	*k2 (Urbana)*	Tan saddle seed coat, near isogenic line of Harosoy
T261	S56-26	*k2 (Columbia 1) Mdh1-n (Columbia 1)*	Tan saddle seed coat and malate dehydrogenase 1 null
	L67-3483 [b]	*k2 (Columbia 2)*	Tan saddle seed coat
T325	CD-3	*Mdh1-n (Ames 4) y20 (Ames 4)*	Yellow foliage and malate dehydrogenase 1 null
	A1937		Parent of *w4-m* mutable line
	X1878		Parent of *w4-m* mutable line
	X2717		Parent of *w4-m* mutable line
	X2937		Parent of *w4-m* mutable line
	Lincoln		Soybean cultivar suspected origin of *Y18-m* mutable allele
	Illini		Soybean cultivar suspected origin of *Y18-m* mutable allele
	Harosoy		Wild type
T334	X-197	*k2 (Urbana) Mdh1-n (Ames 7) y20 (Ames 5)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T335	X-203	*k2 (Urbana) Mdh1-n (Ames 8) y20 (Ames 6)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T336	X-217	*k2 (Urbana) Mdh1-n (Ames 9) y20 (Ames 7)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T337	X-219	*k2 (Urbana) Mdh1-n (Ames 10) y20 (Ames 8)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T338	X-241	*k2 (Urbana) Mdh1-n (Ames 11) y20 (Ames 9)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T339	X-451 [c]	*k2 (Urbana) Mdh1-n (Ames 12) y20 (Ames 10)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T340	M-7-2	*k2 (Columbia 1) Mdh1-n (Columbia 1) y20 (Ames 11)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T341	M-11-4	*k2 (Urbana) Mdh1-n (Ames 13) y20 (Ames 12)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T342	M-11-7	*k2 (Urbana) Mdh1-n (Ames 14) y20 (Ames 13)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T343	M-14-23	*k2 (Urbana) Mdh1-n (Ames 15) y20 (Ames 14)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T344	M-19-3	*k2 (Urbana) Mdh1-n (Ames 16) y20 (Ames 15)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage
T345	M-20-11	*k2 (Urbana) Mdh1-n (Ames 17) y20 (Ames 16)*	Tan saddle seed coat, malate dehydrogenase 1 null and yellow foliage

a : Soybean Genetic Type Collection of USDA-ARS.
b : Soybean Isoline Collection of USDA-ARS.
c : Isolated in F3 generation.

Table 2. Allelism tests at the *Mdh1-n* and the *k2* loci with the new bright yellow mutants by crossing with T261 (*k2 Mdh1-n*) or T239 (*k2*)

Cross	No. of F1 seeds	*Mdh1* pattern	F1 and F2 seed coat
X-197 X T261	2	-[a]	Tan saddle
X-203 X T261	6	-	Tan saddle
X-217 X T239	2	+[b]	Tan saddle
X-217 X T261	2	-	Tan saddle
X-219 X T239	3	+	Tan saddle
X-219 X T261	2	-	Tan saddle
X-241 X T261	5	-	Tan saddle
X-451 [c] X T239	2	+	Tan saddle
X-451 X T261	2	-	Tan saddle
M-7-2 X T261	5	-	Tan saddle
M-11-4 X T261	8	-	Tan saddle
M-11-7 X T261	4	-	Tan saddle
M-14-23 X T261	1	-	Tan saddle
M-19-3 X T261	5	-	Tan saddle
M-20-11 X T261	4	-	Tan saddle

a: malate dehydrogenase 1 null.
b: malate dehydrogenase 1 present.
C : X-451 was isolated in F3 generation from A1937 X T239.

Table 3. Allelism tests at the *y20* locus with the new bright yellow mutants by crossing with T325 (*Mdh1-n y20*) and by intercrosses among new bright yellow mutants

Cross	No. of F1 plants	F1 and F2 phenotype
X-197 X T325	2	Yellow foliage
X-203 X X-219	2	Yellow foliage
T325 X X-203	4	Yellow foliage
T325 X X-217	3	Yellow foliage
X-217 X X-203	2	Yellow foliage
T325 X X-219	1	Yellow foliage
X-241 X T325	2	Yellow foliage
T325 X X-451	1	Yellow foliage
M-7-2 X T325	2	Yellow foliage
T325 X M-11-4	1	Yellow foliage
M-11-7 X X-203	3	Yellow foliage
M-14-23 X T325	2	Yellow foliage
M-19-3 X T325	3	Yellow foliage
M-20-11 X X-203	2	Yellow foliage
M-7-2 X M-20-11	1	Yellow foliage

Table 4. Control crosses of Harosoy with five lines suspected to contain an active transposon

Cross	No. of F2 families	No. of F2 families with yellow mutants
A1937 X Harosoy	144	0
Harosoy X A1937	82	0
X1878 X Harosoy	116	0
Harosoy X X1878	31	0
X2717 X Harosoy	166	0
Harosoy X X2717	20	0
X2937 X Harosoy	100	0
Harosoy X X2937	86	0
Lincoln X Harosoy	138	0
Total	883	0

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Construction of a Soybean Genomic & Root cDNA Library from *Phytophthora* Resistant Line L85-3044.

Introduction

Genomic (lambda, BAC and YAC) and cDNA libraries have been prepared from soybeans, but it is frequently necessary to have a library that is from a specific cultivar. In this respect, we have prepared both a genomic and cDNA library from a soybean line L85-3044 that possesses the *Phytophthora* resistance genes, and this line is a near isogenic line to Williams (Diers et al., 1992). An advantage of these libraries is that they were prepared from size fractionated DNA populations. Consequently the insert size is consistently large. Finally, the clones from the cDNA library were prepared in a pBK-CMV phagemid *in vivo*, thus putative genes can be expressed in both prokaryotic and eukaryotic cells.

Materials and Methods

Plant materials used were soybean line L85-3044, obtained from Dr. R. Bernard from University of Illinois soybean stock center. *E. coli* XL1-Blue MRA was used as the host strain for the genomic library and XL1-Blue MRF was used for construction of the cDNA library. *E coli* strain XLOR and EXAssist helper phage were used for *in vivo* excision of selected lambda clones from the cDNA library and placed into a pBK-CMV phagemid from the ZAP expression vector. For construction of the soybean genomic library from L85-3044, about 10 grams of fresh leaves were harvested from the greenhouse and genomic DNA was carefully isolated according to Doyleís CTAB methods (Doyle, et al., 1990). The quality and the size of DNA was estimated by pulse field gel electrophoresis (CHEF-DRII system, BIO-RAD) in 0.8% GTG agarose (FMC BioProducts, Rockland, ME) and 0.5 x TBE buffer at 10°C for 22 hours at 200 volts. The initial time was 1 second and the switch time was 50 seconds. DNA fragment sizes greater than 200 kb were used for restriction enzyme partial digestion and purified from the gel according to Chen, et al. (1994). About 50 µg of the DNA was digested and extracted with phenol/chloroform (3:1), precipitated with ethanol, and the digested genomic DNA products were incubated with Klenow reagent to fill-in the first two nucleotides of the *Sau*3A-compatible sites (Zabarovsky, et al., 1986). Lambda replacement vector, FIX II/*Xho*1 (Stratagene Inc., La Jolla, CA 92037) was then used for construction of the genomic library and packaged with MaxPlax Lambda Packaging Extract (Epicentre Technologies Inc. Madison, WI 53713) according to manufacture's instruction. The XL1-Blue MAR (P2) was used as the host bacterial strain for the genomic lambda library.

For construction of soybean cDNA root library from L85-3044, soybean seeds were sterilized with chlorine gas and germinated on paper for about a week. The roots were then inoculation with *Phytophthora* race 5 (Smith, et al., 1991). The pathogen isolate *Phytophthora soja* race 5 was provide from Dr. X.B. Yang of Iowa State University. Both inoculated and non-inoculated soybean roots were used for isolation of total RNA by TRIzol reagent (GIBCOBRL Life technologies, Inc. Gaithersburg, MD 20884-9980) according to the manufacture's instruction. The DNase treated total RNA was used for isolation of total mRNA by the PolyATract mRNA isolation system IV (Promega Inc., Madison, WI 53711-5399) according to the manufacture's instructions. The mRNAs isolated from inoculated and non-inoculated soybean roots were pooled for synthesis of cDNA. The cDNAs were synthesized by Super Script Choice system (GibcoBRL Life Technologies Inc.) according to instructions. After synthesis with ^{32}P-dCTP to label the second strand cDNA, the size of cDNA was estimated gel electrophoresis and radiography using Southern blot analysis (Sambrook et al., 1989). The double stranded blunt end cDNAs were ligated to an *Eco*R1 adapter and then phosphorylated by T4 polynucleotide kinase. The phosphorylated ^{32}P-dCTP labeled *Eco*R1-adapted cDNA fragments were size optimized with column chromatography using a cDNA size fraction column (GibcoBRL Life Technologies Inc.). The amount of cDNA from the size fractionation column was determined by the amount of acid-precipitable radioactivity (Cerenkov counts, CMP) of the second strain cDNA from the washed filter. The ligation products and predigested ZAP Express *Eco*R1/CIAP vector DNA were finally packaged into lambda by Gigapack III Gold Packaging Extract (Stratagene Inc.) according to the instruction manual. The host strain XL1-Blue MRFí was used for the cDNA library.

Results and Discussion

To construct a genomic lambda library, high molecular weight DNA must first be isolated free from polysaccharides and other contaminants. Usually, the maximum size of DNA isolated from fresh leaves is about 50 kb or less (Chung et al., 1994) but are poorly suited for preparation of a genomic library (Slightom et al., 1993). We have previously reported a method for isolation of high molecular weight DNA from soybean leaves (Chen, et al., 1994), and this method was used to construct a soybean genomic cosmid library from the *Phytophthora* resistant line L85-3044.

Figure 1 shows the leaf genomic DNA separated by gel electrophoresis. Also shown are the ligation products of soybean L85-3044 genomic DNA *Sau*3A partial digestion to the lambda vector. Lanes 1 to 5 are from CHEF gel electrophoresis of soybean DNA in 0.8% GTG agarose. Lane 1 is the lambda concatemers used as DNA molecular weight size standards. Only DNA with fragments greater than 200 kb (lane 5) were used for construction the library. Lane 6 shows the FIX II/*Xho*1 vector without ligation and lane 7 and 8 are the ligation products of partially digested DNA and vector. The average number of recombinant phages of this lambda genomic library is 8.5×10^5. According to Slightomís calculation the theoretical recombinant phage numbers for a soybean genomic library should not less

than 2.4 x 10^5 if 0.99% probability is desired. The average insert size is about 17 kb and the soybean genomic size is 8.7 x 10^8 bp (Slightom et al., 1993). We isolated 20 positive lambda clones from this library by screening with a single probe from 7 plates (about 45,000 plaques per plate) in one of our experiments.

Another genomic library of L85-3044 was also constructed with lambda GEM-12 *Xho*1 half-site arm vector (Promega, Inc.). However, the packaged phage titration of this library was significantly lower than the lambda FIX II/*Xho*1 library. Both bacterial host strains were *mcr*A- and *mcr*B-, allowing propagation of genomic DNA clones containing methylated cytosine residues, but, the bacterial strain used for Lambda GEM-12 *Xho*1 half-site vector was *rec*D-, which reduces recombinational loss but does allow certain eukaryotic clones to grow. Thus, this may results in a lower clone titration compared with the library constructed with Lambda FIX II/*Xho*1 vector. The constructed soybean genomic library from line L85-0344 from lambda FIX II/*Xho*1 vector is sensitive to P2 inhibition selection, that is, only recombinant phage grow on bacterial strains containing the P2 phage. This is due to the fact that wild type lambda phage contain active *red* and *gam* genes on the stuffer fragment and are unable to grow on host bacterial strains that contain P2 phage lysogens. This characteristic significantly reduces the background of non-recombinant phages in the library. In addition, the lambda FIX II polylinker used in this library allows the isolation of flanking T3 and T3 promoters as an intact cassette after digestion with *Not*1. The T3 and T7 promoters flanking the insertion sites can also be used to generate end-specific probes for used in chromosome walking and restriction mapping. Figure 3 shows the flanking restriction enzymes sites around the *Xho* I site that soybean genomic DNA fragments were ligated into the Xho 1 partial fill in site in lambda FIX vector.

For a Soybean L85-3044 root cDNA library it is important to have large sized fragments. The size of cDNA after second strand synthesis was determined gel electrophoresis and radiography through Southern blot analysis. The data in Figure 2 show the Southern blot analysis of the pooled random samples of ^{32}P-dCTP labeled second strand cDNAs before size fraction column chromatography. The cDNA fragments range from 0.5 to 7 kb with the major fraction ranging from 1 to 4 kb. To avoid incorporating of small sized cDNA fragments and *Eco*R1 adapters into the cDNA library, we used size fractionation column chromatography to purify cDNA ligation products. The phosphorylated *Eco*R1-adapted cDNA fractions were ligated into our lambda vector (predigested ZAP Express *Eco*R1/CIAP DNA) for packing this cDNA library. We observed an average phage number of 3.6 x 10^5. The theoretical phage number should not be less than 1.8 x 10^5 if we assume a 0.99% probability. We isolated 20 positive cDNA clones from this cDNA library by screening with a single probe from 8 plates (about 45,000 plaques per plate) in one of our experiments. And, the fragment size of the cDNA inserts ranged from 0.8 to 3.4 kb in this experiment.

Purified clones resulting from a screen of this cDNA library, as well as random pooled clones from this library, can be excised into a pBK-CMV phagemid *in vivo* using the interference resistant helper phage XLOLR (Stratagene Inc.). Figure 4 shows where the soybean cDNA library was cloned into the *Eco*R1 site in the multiple cloning site (MCS). The insertions in pBK-CMV can be expressed in both prokaryotic and eukaryotic cells because the vector contains both the *lac*Z promoter for prokaryotic expression and CMV promoter for eukaryotic expression. The gene product (fusion protein of β-galactosidase) of the selected clone or random pooled clones of this cDNA library can be expressed in *E coli* by adding 0.2 to 1 mM of IPTG in the liquid LB culture. We checked the expression of the random pooled cDNA clone samples from this cDNA library in *E coli* and observed 16 blue clones out of 210 white recombinant expression clones (92.4% expression of recombinants). To increase the expression level in eukaryotic cells, the *lac*Z promoter can be removed by *Nhe*I and *Spe*I digestion of the excised phagemid, followed by re-legating of the intact cassette and then selecting clones which have lost the 200 bp promoter fragment. The eukaryotic expression function of clone(s) of this library allows either transient or stable transfer of selected clones (target genes) directly into mutant or complementary cells or tissue for verification of the gene candidates.

Acknowledgments

We would like to thank Mr. Jinbo Zhang of Iowa State University for technical assistance in the construction of the genomic library. This work was supported by a grant to Alan Atherly by the United Soybean Board.

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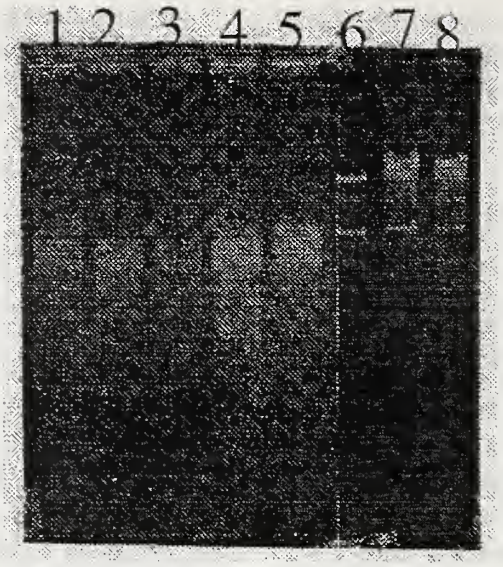

Figure 1. CHEF gel electrophoresis of soybean total genomic DNA isolated from leaves as well as the ligation products of partially digested genomic DNA. Lanes 1 to 5 are from soybean total genomic DNA in 0.8% GTG agarose. Lane 1 is the lambda concatemers used as DNA molecular weight size standards. Lanes 6 to 8 are 1% agarose gel separated ligation products of soybean L85-3044 genomic DNA *Sau*3A partial digestion products to the lambda vector. Line 6 shows the FIX II/*Xho* 1 vector without ligation and Lane 7 and 8 are the ligation products of partially digested DNA and the vector.

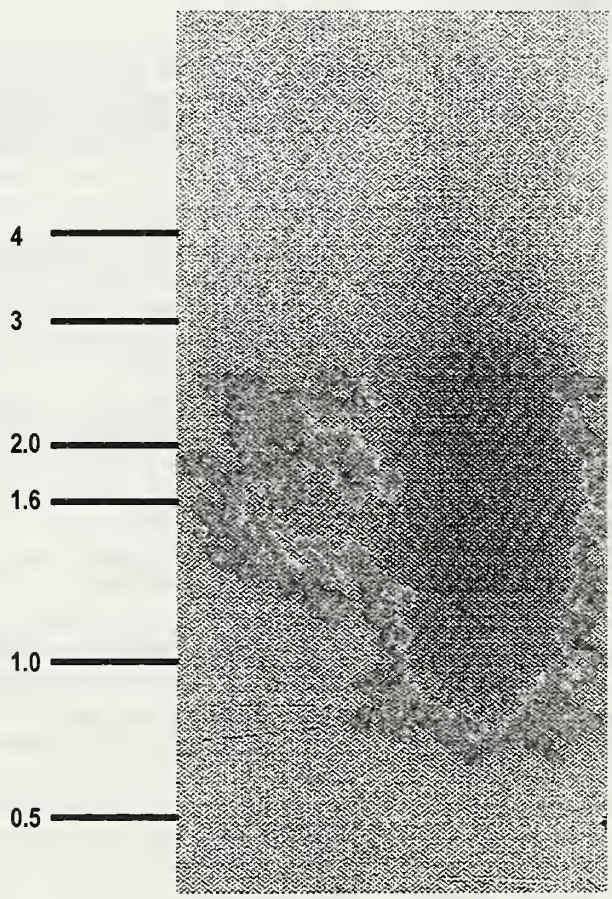

Figure 2. Southern blot analysis of the pooled random samples of ^{32}P-dCTP labeled cDNAs before size fractionation with column chromatography. The cDNA fragments range from 0.5 to 7 kb with the major fraction ranging from 1 to 4 kb.

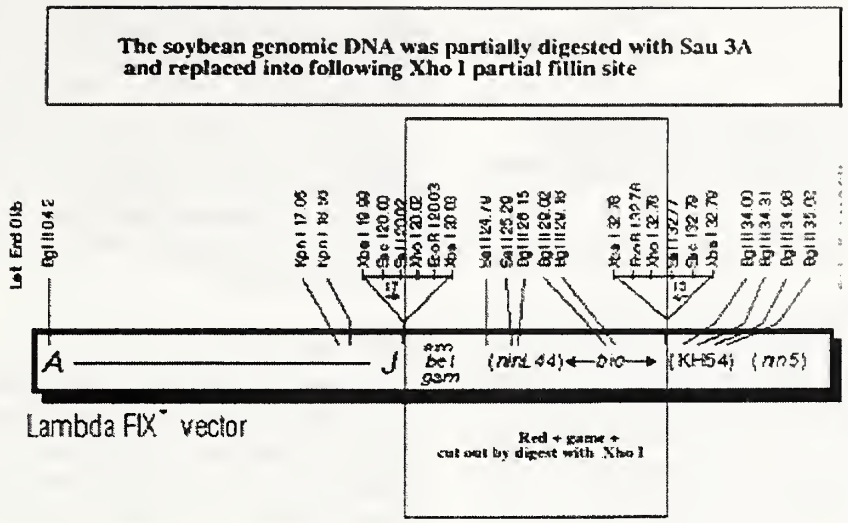

Figure 3. The soybean genomic DNA was partially digested with *Sau* 3A and replaced into *Xho I* partial fill in site. The restriction enzymes flanking around the *Xho I* are the enzymes that can cut soybean genomic insert fragment out from the Lambda FIX vector.

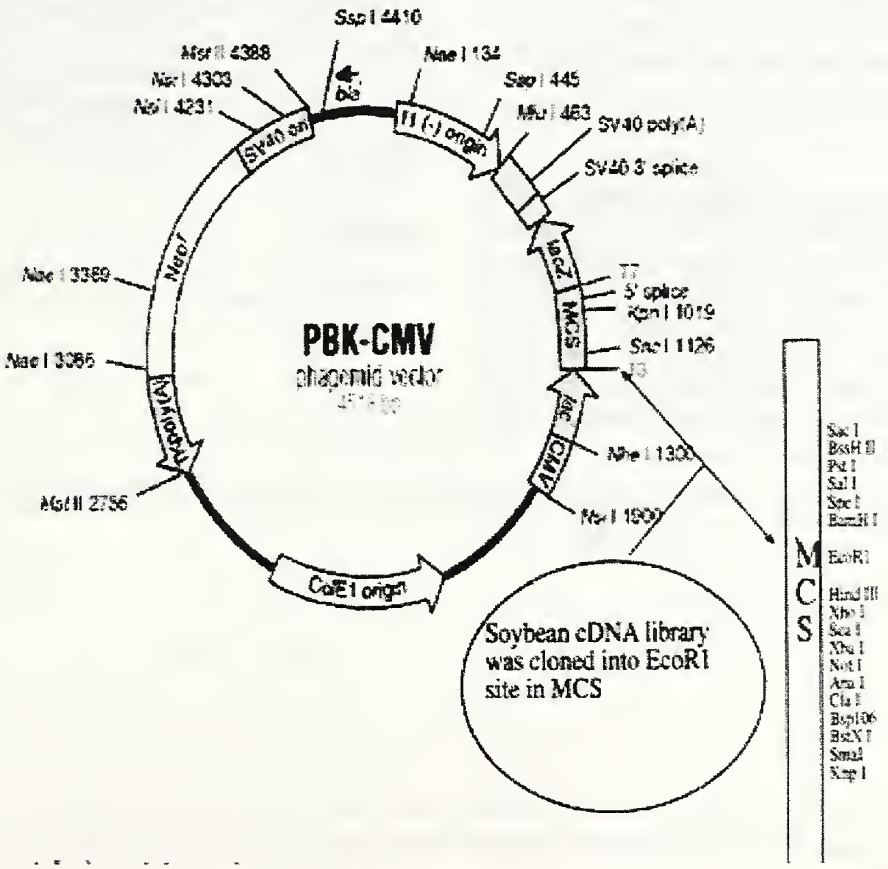

Figure 4. It showed that soybean cDNA library was cloned into the EcoR1 site in the multiple cloning site (MCS). The positions of restriction enzyme cutting sites, promoters and terminators of lac Z and CMV, replication origins of f1, CoE1 and SV40, lacZ gene, SV40 poly A terminate signal, and selection gene G418 for neomycin/kanamycine resistance were also illustrated in the map. This figure was adapted based on the pMK-CMV phagemid map of Stratagene Inc.

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Further Genetic Characterization of a Duplicate-Factor Male-sterile, Female-sterile Trait in Soybean

Introduction

Last year we reported on the origin and genetic characterization and identification of the two different loci of the duplicate-factor male-sterile, female-sterile phenotype (Palmer and Lewers 1997). The parent plants used in cross-pollinations had been individually numbered and were progeny tested to distinguish homozygous dominant genotypes from heterozygous genotypes. Data were reported that only included parents where both male and female plants had been identified individually. Thus the genotypes of both parents were known and the F_2 data were organized and interpreted based upon this knowledge.

To expedite the large number of cross-pollinations that needed to be obtained to answer our objectives, for most cross-pollinations only the identity of the individual male parents was maintained. Our objective was to confirm the identity of the two different loci of the duplicate-factor male-sterile, female-sterile phenotype.

Results

The intercrosses between families of identical genotype gave 512 fertile: 67 sterile F_1 plants ($X^2$8:1 = 0:12, P = 0.73). The F_2 family segregation for nonsegregating: (3:1) from cross-pollination of fertile plants (AAbb or Aabb) x homozygous dominant fertile plants (AAbb) fit the expected 2:1 ratio (Table 1). And the F_2 family segregation for nonsegregating:(3:1) from cross-pollinations of fertile plants (AAbb or Aabb) x heterozygous (Aabb) fertile plants fit the expected 2:3 ratio (Table 1). Within segregating families both cross-pollination combinations fit the expected 3 fertile: 1 sterile ratio (Table 2).

The intercrosses between families of different genotypes gave 709 fertile:89 sterile F_1 plants ($X^2$8:1 = 0.12, P = 0.73). The F_2 family segregation for 3:1 to 15:1 from cross-pollinations of fertile plants (AAbb orAabb) x homozygous dominant fertile plants (aaBB) fit the expected 1:2 ratio (Table 3). And the F_2 family segregation for 3:1 to 15:1 from cross-pollinations of fertile plants (AAbb or Aabb) x heterozygous dominant fertile plants (aaBb) fit the expected 3:2 ratio (Table 3). Within segregating families both cross-pollination combinations fit the expected 3:1 or 15:1 ratios (Table 4).

Discussion

Our hypothesis was that we had a duplicate-factor trait for male sterility, female sterility. The data given last year (Palmer and Lewers 1997) included only F_2 families where each parent was confirmed by progeny testing as heterozygous for male sterility. These data based upon few F_2 families strongly suggested that the two different loci of the duplicate-factor phenotype were identified.

Both the F_2 family data and the number of F_2 plants within segregating combinations from the 1997 data confirmed our previous designations of the two different genotypes. Allelism tests of these two different male-sterile, female-sterile loci were made in summer 1997 with the known mutants; *st2, st3, st4, st5,* and *st6st7*. F_1 seed will be advanced to F_2 seed in Puerto Rico. The F_2 classification will be made in Ames in summer 1998. Gene symbols will be assigned after the data have been analyzed.

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Table 1. Identical genotypes: Number of F_2 families from intercrosses among fertile plants from families segregating 3 fertile: 1 sterile plant.

Cross-pollination combinations[a]	No. F_2 families: All fertile	3:1	X^2 (2:1)[b]	P	X^2 (2:3)[c]	P
(AAbb x AAbb / Aabb x AAbb)	137	70	0.02	0.89		
(AAbb x Aabb / Aabb x Aabb)	123	155			2.08	0.15

[a] Similar results are expected for number of F_2 families and within segregating combinations if the crosses involve aaBB and aaBb and if the cross-pollinations occur in equal frequencies and if loci A and B are not linked.
[b] Expected F_2 frequency and F_3 phenotype; 2 (all fertile) : 1 (3 fertile: 1 sterile).
[c] Expected F_2 frequency and F_3 phenotype; 2 (all fertile) : 3 (3 fertile: 1 sterile).

Table 2. Identical genotypes: Number of F_2 plants within segregating combinations from intercrosses among fertile plants from families segregating 3 fertile: 1 sterile plant.

Cross-pollination combinations[a]	No. F_2 plants		X^2 (3:1)	P
	Fertile	Sterile		
Aabb x AAbb	5338	1749	0.39	0.53
(AAbb x Aabb / Aabb x Aabb)	11,985	3941	0.55	0.46

[a] Similar results are expected for number of F_2 plants if the crosses involve aaBB and aaBb and if the crosses occur in equal frequencies and if loci A and B are not linked.

Table 3. Different genotypes: Number of F_2 families from intercrosses among fertile plants from families segregating 3 fertile: 1 sterile plant.

Cross-pollination combinations	No. F_2 families					
	3:1	15:1	X^2 (1:2)[a]	P	X^2 (3:2)[b]	P
(AAbb x aaBB / Aabb x aaBB)	90	155	1.27	0.26		
(AAbb x aaBb / Aabb x aaBb)	239	150			0.33	0.57

[a] Expected F_2 frequency and F_3 phenotype; 1 (3 fertile: 1 sterile): 2 (15 fertile: 1 sterile).
[b] Expected F_2 frequency and F_3 phenotype; 3 (fertile: 1 sterile): 2 (15 fertile: 1 sterile).

Table 4. Different genotypes: Number of F_2 plants within segregating combinations from intercrosses among fertile plants from families segregating 3 fertile: 1 sterile plant.

Cross-pollination combinations	No. F_2 plants		X^2 (3:1)	P	No. F_2 plants		X^2 (15:1)	P
	Fertile	Sterile			Fertile	Sterile		
(AAbb x aaBB / Aabb x aaBB)	6434	2064	2.29	0.13	14,061	942	0.02	0.89
(AAbb x aaBb / Aabb x aaBb)	17,539	5783	0.52	0.47	13,395	881	0.15	0.70

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Genetic Linkage in Soybean: Classical Linkage Groups 6 and 8

Classical Linkage Group 6 (LG6) is defined by two known gene loci; *Df2* and *Y11*, and classical Linkage Group 8 (LG8) is defined by seven known gene loci; *W1, Wm, Ms1, Adh1, Ms6,* and *Y23.*

Genetic evidence suggests that a chromosome interchange (translocation) from *Glycine soja* (Sieb. & Zucc.) (Palmer and Heer 1984), may be linked to mutants of LG6 and LG8 (Palmer 1985). It was not determined whether the same chromosome was involved with the two linkage groups. It is possible that two or more linkage groups may in fact be the same linkage group. Additional studies are needed to substantiate and complete the reported linkages.

This report presents F2:3 linkage data on loci *Df2, Y11, Ms1,* and *W1* in the coupling phase.

Materials and Methods

The genetic line *w1 w1 df2 df2 Ms1 ___ Y11 y11* was derived by crossing several genetic stocks and selecting the desired genotype. This genotype usually is grown in the glasshouse where the dwarf (*df2*) trait is not so extreme. Cross-pollinations can be made by using the genetic line as female parent. The male parent was Triplo 13 (primary trisome for chromosome 13) (Xu et al. 1997).

The five 40-chromosome F1 plants were grown in the glasshouse in summer 1995 and threshed individually. The F2 seed were planted at the Bruner Farm near Ames, Iowa in summer 1996. The tall fertile plants were threshed individually. Sixty seed from each tall fertile F2 plant were tractor planted in summer 1997 at the Bruner Farm. Data were collected on a F2:3 progeny basis for flower color, plant color, plant height, and fertility. The data were analyzed by using a computer program, based upon the maximum likelihood method (Allard 1956), developed by Dr. X. F. Chen (personal communication) at Iowa State University.

Results and Discussion

A total of five F1 seed were obtained from the cross of *w1 w1 df2 df2 Ms1 ___ Y11 y11* by Triplo 13. Three F1 plants were green (*Y11 Y11*) and two were yellow green (*Y11 y11*). Tall fertile F2 plants were threshed individually and evaluated as F2:3 progeny rows. Data were combined across F2:3 families and arranged as two-point linkage determinations (Table 1). The *W1-Ms1* recombination value (26.9 ± 2.6) is similar to the consensus value (30) reported by Palmer and Hedges (1993). The *Df2-Y11* recombination value (24.7 ± 2.2) is twice the consensus value (12) reported by Weiss (1970). His data were from crosses in repulsion phase while the present data were obtained in coupling phase.

The other four two-point linkage tests have not been reported previously. The *Ms1-Df2* recombination value (35.3 ± 3.0) probably represents a genetic linkage. The remaining two-point combinations may represent very loose linkage or represent independent assortment.

The definitive experiment would be to use the primary trisome (Triplo 13) for linkage group 8 and test for linkage with mutants for linkage group 6. F1 seed have been produced with a combination of mutants of linkage groups 6 and 8 as one parent and Triplo 13 as the other parent. A comparison of F2 recombination values from self-pollinated progenies of 40-chromosome F1 and from 41-chromosome F1 plants, should provide the evidence to determine if linkage group 6 and linkage group 8 are the same chromosome or two different chromosomes.

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Figure 1. Recombination values for gene loci associated with soybean classical Linkage Groups 6 and 8; F2:3 data.

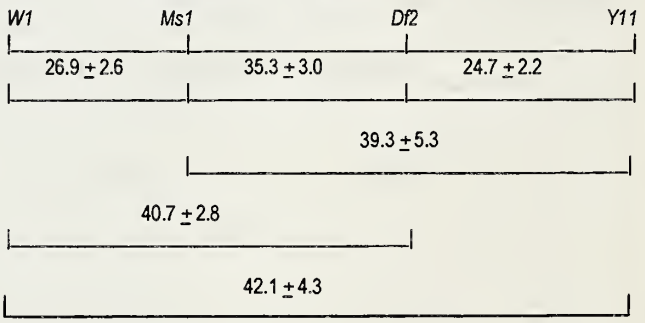

Table 1. Linkage relationships for gene loci from soybean classical Linkage Group 6 and Linkage Group 8; F2:3 data.

Gene pair	AABB	AABb	AaBB	AaBb	χ^2(1:2:2:4)	R± SE
W1-Df2	104	102	166	202	53.14	40.7 ± 2.8
W1-Ms1	73	35	58	121	66.85	26.9 ± 2.6
W1-Y11	50	52	85	78	40.17	42.1 ± 4.3
Ms1-Df2	83	93	58	116	68.10	35.3 ± 3.0
Ms1-Y11	32	47	26	45	28.37	39.3 ± 5.3
Y11-Df2	117	56	53	116	184.99	24.7 ± 2.2

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Genetic Linkage in Soybean: Classical Linkage Groups 6 and 8 and 'Clark' Translocation.

Classical genetic linkage mapping in soybean has mostly involved intercrossing genotypes differing in two or more qualitative Mendelian traits. The order of loci have been determined using recombination values estimated from segregation data collected from F2 populations and/or F2:3 families. The advantages in the use of translocations over regular Mendelian genes in linkage mapping have been documented. The first evidence of the use of translocations to locate a gene to a chromosome was by Palmer (1976). Thereafter, Sadanaga (1983), Sadanaga and Grindeland (1984), and Sacks and Sadanaga (1984) have reported linkage studies that involved the use of translocations. Palmer (1985) presented genetic evidence that suggested that the chromosomal interchange (translocation) in Clark T/T may be linked to mutants of LG6 and LG8. Two gene loci, *Df2* and *Y11*, define classical linkage group 6. Linkage group 8 is one of the well-studied linkage groups, and is defined by seven known gene loci; *W1*, *Wm*, *Ms1*, *St5*, *Adh1*, *Ms6*, and *Y23*. Using three-point linkage tests, Lewers and Palmer (1993) determined the order of loci of LG8. This report presents recombination values based upon F2 data from crosses involving the 'Clark' translocation.

Materials and Methods

The genetic line *w1w1df2df2Ms1_Y11y11* (Palmer and Chen 1998) was used as female parent (*w1w1df2df2ms1ms1Y11y11NN*) in crosses with Clark T/T (*W1W1Df2df2Ms1Ms1Y11Y11TT*) in the greenhouse. Clark T/T is near-isogenic Clark that is homozygous for a translocation derived through backcrossing with PI 101404B *Glycine soja* (Sieb. & Zucc.) (Palmer and Heer 1984). The F1s were grown in Puerto Rico in winter 1996, and the F2 populations grown at the Bruner Farm near Ames, Iowa in summer 1997. The F2 populations were scored and data collected on flower color, plant height, plant color, fertility (*Ms1* locus), and semisterility (translocation heterozygote). To determine fertility/semisterility of dwarf plants, pollen samples were taken from each plant separately in vials with 70% ethanol. Pollen grains were stained in I_2KI solution, and counted under the microscope at 400x magnification. A total of 200 pollen grains per sample were counted to determine fertile (all 200 grains stained golden brown), sterile (none stained, that is, yellow, shrunken grains), and semisterile (approximately 1:1 stained to none stained grains) plants. The data reported here are from populations that did not segregate for plant color, that is, were *Y11 Y11*. The data were analyzed using LINKGAE-1 (Suiter et al, 1983), and a computer program based on the maximum likelihood method (Allard 1956), developed by Dr. X. F. Chen (personal communication) at Iowa State University. Linkage estimates between the interchange breakpoint and the other loci were calculated following the method of Hanson and Kramer (1950).

Results and Discussion

Data were collected and combined from a total of eleven populations that did not segregate for plant color. Two-point linkage values were calculated, and are presented in Table 1. The recombination value for *W1-Ms1* is 30.3 ± 2.5%. This value agrees with the consensus value (30) reported by Palmer and Hedges (1993). Recombination values between *W1-Df2*, and *Ms1-Df2* are 37.3 ± 2.4, and 28.5 ± 2.5% respectively, suggesting genetic linkage similar to the report by Palmer and Chen (1998). The recombination value between the flower color locus, *W1*, and the translocation breakpoint is 44.5 ± 12.2%. This may represent independent assortment, similar to the report by Palmer (1976). *Ms1* and *Df2* are fairly closely linked to the breakpoint (recombination values of 7.1 ± 2.7%, and 19.6 ± 2.2% respectively), the value of 7.1 for *Ms1* being similar to that reported by Palmer (1976). These recombination values are further indications of linkage (Figure 1). Thus LG6 and LG8 may actually be the same linkage group.

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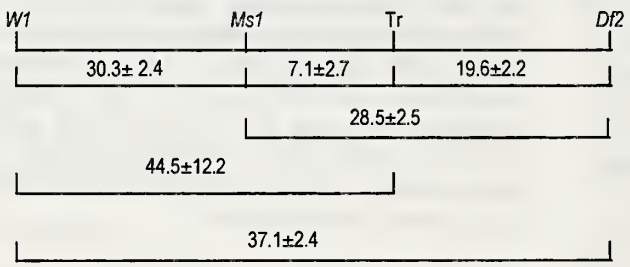
Table 1. Linkage relationships of gene loci of classical linkage groups 6 and 8, and a translocation breakpoint in Clark T/T; F2 data.

Gene pair	A-B-	A-bb	aaB-	aabb	χ^2(9:3:3:1)	R ± SE†
W1-Df2	765‡	193	206	133	48.5	37.1 ± 2.4
W1-Ms1	838	111	209	139	130.6	30.3 ± 2.5
Ms1-Df2	865	115	182	135	146.5	28.5 ± 2.5
	ASS	AN	aSS	aN§		
W1-Tr¶	517	321	135	74	0.1	44.5 ±12.2
Ms1-Tr	652	395	0	0	63.1	7.1 ± 2.7
Df2-Tr	570	295	82	100	34.5	19.6 ± 2.2

†, R ± SE = recombination value ± standard error;
‡, 765 = number of plants;
§, SS = semisterile, N = fertile;
¶, Tr = interchange breakpoint.

Figure 1. Recombination values for gene loci of classical linkage groups 6 and 8 in relation to a translocation breakpoint in Clark T/T; F2 data.



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Genetic Linkage in Soybean: the *Y23* and *St5* Loci

Introduction

Classical linkage group 8 is defined by seven known gene loci; *W1, Wm, Ms1, St5, Adh1, Ms6,* and *Y23.* In a comprehensive linkage study involving six of the loci (all but *Wm*) in three-point linkage tests, Lewers and Palmer, (1993), determined gene order based upon recombination values. Their tests, however, did not distinguish the order of the *St5, Y23,* and *Adh1* loci relative to *W1.*

The present study has identified the genotype *w1 w1 adh1 adh1 y23 y23 St5 st5.* This genetic stock will be used in cross-pollinations with cultivar BSR 101 (Tachibana et al. 1987) to obtain linkage estimates in coupling phase of the four loci.

This report presents recombination values based upon F2:3 progeny tests for the *Y23-St5* gene pair.

Materials and Methods

The original cross was:

Y23 Y23 w1 w1 adh1 adh1 St5 - x *y23 y23 w1 w1 adh1 adh1 St5 St5*

The male-parent plants were individually identified and progeny tested to discriminate the fertile *St5 St5* genotype from the fertile *St5 st5* genotype. The *st5 st5* genotype is male sterile and female sterile.

In the F2 generation, all fertile plants (green foliage and yellow foliage) were single-plant threshed, F2:3 progeny rows were classified in summer 1997.

Results and Discussion

A total of 106 F1 plants were single-plant threshed. Progeny testing identified male parents that were *St5 St5* or St_5 st_5. In the F2 generation from the *St5 St5* cross-combinations, the data for number of F2 families were: 52 all green: 107 segregating green/yellow: 48 all yellow. The data fit the expected ratio, χ^2 (1:2:1) = 0.39, P = 0.82. In heterozygous F1 plants, the *Y23* and *y23* gametes were transmitted in equal frequency. In the F2 generation from the *St5 st5* cross-combinations, 55 F2 progenies were all fertile plants and 51 F2 progenies segregated fertile/sterile plants. The data indicate that the *St5* gamete and the *st5* gamete were transmitted in equal frequency (χ^2 (1:1) = 0.15; P = 0.70).

Within families segregating both fertile/sterile and green/yellow plants, 863 fertile F2 plants were single-plant threshed. The data were 659 green : 204 yellow plants, a good fit to the expected ratio (χ^2 (3:1) = 0.85; P = 0.36).

Progeny of the green F2 plants did not fit the segregation expected for two unlinked loci (χ^2 1:2:2:4 = 558.86; P = 0.00) (Table 1). A recombination value of 6.12 $\pm$ 0.69 was calculated for the *Y23* and *St5* loci (Allard, 1956). Lewers and Palmer (1993) reported F2 recombination values of 0.96 $\pm$ 0.45 and 2.37 $\pm$ 1.15 and a F2:3 value of 1.01, based upon 147 F2 families. Palmer et al. (1990) reported a F2:3 recombination value of 7.2 $\pm$ 1.7, based upon 79 F2 families. Our F2:3 data recombination value of 6.12 $\pm$ 0.69 is similar to reported values. All previous values plus this value are from alleles in the repulsion phase.

Of the 204 all yellow F2:3 progenies, only 9 families were segregating fertile/sterile plants (Table 1). Recombination studies will use this newly derived *w1 w1 adh1 adh1 y23 y23 St5 st5* genetic stock in cross-pollinations to obtain recombination values based upon coupling phase data. These data will be used to establish gene order for classical linkage group 8 in soybean.

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Table 1. Phenotype and number of F2:3 progeny rows for the *Y23* and *St5* linkage test

Phenotype	No. F2 families
All green, all fertile	13
All green, seg. fertile/sterile	15
Seg. green/yellow, all fertile	37
Seg. green/yellow, seg. fertile/sterile	594
All yellow, all fertile	195
All yellow, seg. fertile/sterile	9

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Inheritance and Allelism of an EMS-Generated Necrotic Root Mutant

Four recessive allelic necrotic root mutants descended from germinal revertants in the *w4*-mutable line of *Glycine max* (L.) Merr. have been identified (Kosslak et al., 1996; Andersen and Palmer, 1997). These mutants arose from separate genetic events and three have been assigned gene symbols and Genetic Type Collection numbers; *m* (Ames 1) is T328H, *m* (Ames 2) is T329H, and *m* (Ames 3) is T330H (Kosslak et al. 1997). The fourth necrotic root mutant was called 'unknown necrotic root' mutant (Andersen and Palmer, 1997).

Two necrotic root plants were identified in family EMS-95 which were derived from EMS treated cultivar AgriPro 1776. The objectives were to; 1) characterize the inheritance of the EMS-induced necrotic root mutant, and 2) determine the allelism of the EMS-induced necrotic root mutant with the four previously described necrotic root mutants.

Materials and Methods

A soybean mutagenesis study with EMS treatment of cultivar AgriPro 1776 was initiated to obtain root mutants. Treatment was based upon the protocol of Hammond and Fehr (1984). The two necrotic root mutants were identified in family EMS-95.

The two necrotic root plants were grown in the glasshouse and cross-pollinations to AgriPro 1776 and to cultivar Harosoy were made. The F1 plants of the sibling crosses were used as male parents in cross-pollinations with the four known allelic necrotic root mutants. For some female parent plants, the identity of the plants used in cross-pollinations were maintained. Progeny of these self-pollinated plants distinguished between homozygous dominant and heterozygous necrotic root plants.

The F1 sibling plants were allowed to self-pollinate and inheritance was determined in the F2 generation. These sibling crosses also served to maintain the necrotic root trait in the original AgriPro 1776 germplasm.

The EMS-95 necrotic root plants also were cross-pollinated to cultivar Harosoy. The F2 generation gave us data on the inheritance of the necrotic root trait in an unrelated genetic background.

The F1 and F2 seed from all cross-pollinations were planted on germination paper. The seedlings were scored for root necrosis 10 days after planting. The F2 families were scored as either segregating the necrotic root phenotype or nonsegregating. The results were analyzed for statistical significance by using a Chi-square test.

Results and Discussion

Two necrotic root plants were identified in family EMS-95 in the M2 generation. These plants were used as male parents in crosses to the non-mutagenized parent cultivar AgriPro 1776. The self-pollination of these F1 plants yielded 3 normal root: 1 necrotic root plant (501:160, χ^2 3:1 = 0.22, P = 0.68). The necrotic root mutant segregated as a single-gene recessive. Homozygous recessive necrotic root plants are extremely weak and if they survive in the glasshouse only produce one to two seeds. Seed from this sibling cross is used to maintain the mutant as the heterozygous genotype.

The cross-pollinations of known heterozygotes with cultivar Harosoy gave F2 families of 5 nonsegregating: 4 segregating necrotic root (χ^2 1:1 = 0.11, P = 0.74). Within segregating families, the data fit the expected 3 normal root: 1 necrotic plant (444:141, χ^2 3:1 = 0.25, P = 0.62), confirming single gene recessive inheritance.

The F1 data from the allelism tests of the four allelic necrotic root mutants with the known EMS-95 heterozygotes gave both normal root and necrotic root plants. In the four cross combinations of non-genotyped plants x known EMS-95 heterozygotes, the F1 ratio was 5 normal root : 1 necrotic root plant (Table 1). In crosses between heterozygous plants, the F1 ratio was 3 normal root : 1 necrotic root plant (Table 1).

The F2 data from the allelism tests were collected only from the crosses between heterozygous plants. The F2 family segregation was the expected 2 segregating: 1 nonsegregating (Table 1). Within segregating F2 families, the ratio was 3 normal root : 1 necrotic root plant (Table 1).

The F1 and F2 data confirmed that the EMS-95 necrotic root mutant is allelic to the four known necrotic root mutants identified in gene-tagging studies (Palmer et al., 1989; Andersen and Palmer, 1997).

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Table 1. Allelism tests of unknown EMS-induced necrotic root mutant with four necrotic root mutants in soybean; F1 and F2 data.

Cross	No. F1 plants						No. F2 families				No. F2 plants			
	Non-mutant	Necrotic	χ^2(5:1)	P	χ^2(3:1)	P	Seg.	Nonseg.	χ^2(2:1)	P	Non-mutant	Necrotic	χ^2(3:1)	P
*Rn_* (Ames 1) x EMS-95 (het.)	25	4	0.17	0.68										
Rn m (Ames 1) x EMS-95 (het.)	6	2			0.0	1.00	5	1	0.75	0.39	417	147	0.34	0.56
*Rn_* (Ames 2) x EMS-95 (het.)	30	5	0.14	0.70										
Rn m (Ames 2) x EMS-95 (het.)	9	2			0.27	0.60	5	4	0.50	0.48	401	120	1.08	0.30
*Rn_* (Ames 3) x EMS-95 (het.)	32	6	0.02	0.88										
Rn m (Ames 3) x EMS-95 (het.)	15	4			0.16	0.69	8	3	0.18	0.67	673	239	0.71	0.40
*Rn_* (unknown) x EMS-95 (het.)	23	4	0.07	0.80										
Rn m (unknown) x EMS-95 (het.)	23	7			0.04	0.83	12	8	0.40	0.52	948	292	1.39	0.24

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Linkage Analysis of a Nonfluorescent Root Mutant (fr_2) with Five Isozymes in Soybean

Introduction

In nonmutant soybean [*Glycine max* (L.) Merr.], root fluorescence is observed upon exposure to ultraviolet light. Nonfluorescence of soybean roots is controlled by loci, fr_1, fr_2, Fr_3, fr_4, and fr_5 (Torkelson and Palmer, 1997). This trait has been a useful marker in plant genetics, in tissue culture research, and in genetic diversity analyses (Sawada and Palmer, 1987). Similarly, isozyme variants have been valuable markers in widespread comparisons of protein inheritance and genetic diversity analyses. These markers have supported development of traditional linkage maps and investigations of polymorphism. In this study, our objective was to establish the presence, or absence, of genetic linkage between five isozymes, *Dia*, *Idh*, *Pgd*, *Pgi*, and *Pgm* , and one of the five root fluorescence loci, fr_2.

Materials and Methods

The nonfluorescent seeds tested for this analysis were derived from seed mutagenized by ethylmethane sulfonate (EMS) events. A96-663 and -664 occurred as independent mutations.

One hundred and fifty seeds from crosses of cultivar Evans X A96-663 and -664 were scarified and placed on moist germination paper, and allowed to incubate in growth chamber conditions for two days. Samples for isozyme analysis were extracted from cotyledon tissue using a Drummond microdispenser (3 mm dia.) and placed into microcentrifuge tubes with 4 µl of sucrose homogenization buffer. The seeds continued to grow for 10 days until the ultraviolet light detection method was used to distinguish between fluorescent and nonfluorescent roots.

Isozymes of the prepared samples were elucidated on three gel systems by horizontal starch gel electrophoresis (Cardy and Beversdorf, 1984). Diaphorase (*Dia*, EC 1.8.1.4), 6-phosphogluconate dehydrogenase (*Pgd*, EC 1.1.1.44), and phosphoglucomutase (*Pgm*, EC 5.4.2.2) were interpreted on the Ct-gel system, while phosphoglucose isomerase (*Pgi*, EC 5.3.1.9) and isocitrate dehydrogenase (*Idh*, EC 1.1.1.42) were defined on the B- and D-gel systems, respectively (Palmer, 1997).

Results and Discussion

No linkage was found between the fr_2 locus and four of the five isozymes under inspection. However, a X^2 value for fr_2-*Idh* indicates the possibility of linkage (Table 1). The combined X^2 value for fr_2-*Idh* deviates from the other fr_2-isozyme combinations, and shows greater than 95% significance (P = 0.04). The recombination value was 42.0 +/- 4.0.

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Table 1. Linkage analysis of the fr_2 (root fluorescence) locus with five isozymes in soybean; F_2 data.

Genetic combination: Evans X A96-663					
Isozyme	Expected Frequency (Isozyme X fr_2 phenotype)	X^2	df	P	R +/- S
Dia	3:1:6:2:3:1	0.67	2	0.72	46.0 +/- 5.0
Idh	3:1:6:2:3:1	1.57	2	0.46	44.0 +/- 5.0
Pgd	3:1:6:2:3:1	0.95	2	0.62	49.0 +/- 5.0
Pgi	9:3:3:1	0.58	1	0.45	50.0 +/- 7.0
Pgm	9:3:3:1	1.11	1	0.29	42.0 +/- 7.0
Genetic combination: Evans X A96-664					
Isozyme	Expected Frequency (Isozyme X fr_2 phenotype)	X^2	df	P	R +/- SE
Dia	3:1:6:2:3:1	0.32	2	0.85	47.0 +/- 5.0
Idh	3:1:6:2:3:1	5.61	2	0.06	41.0 +/- 5.0
Pgd	3:1:6:2:3:1	0.57	2	0.75	48.0 +/- 5.0
Pgi	9:3:3:1	0.12	1	0.73	49.0 +/- 6.0
Pgm	9:3:3:1	0.01	1	0.94	50.0 +/- 6.0
Combined data: Evans X A96-663 + A96-664					
Isoenzyme	Expected Frequency (Isozyme X fr_2 phenotype)	X^2	df	P	R +/- SE
Dia	3:1:6:2:3:1	0.98	2	0.61	47.0 +/- 4.0
Idh	3:1:6:2:3:1	6.68	2	0.04	42.0 +/- 4.0
Pgd	3:1:6:2:3:1	1.38	2	0.50	48.0 +/- 4.0
Pgi	9:3:3:1	0.05	1	0.82	49.0 +/- 5.0
Pgm	9:3:3:1	0.55	1	0.46	46.0 +/- 5.0

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Linkage Studies with Necrotic Root Mutants

In soybean [*Glycine max* (L.) Merr.] three necrotic root mutants were identified among germinal revertant progeny of the *w4*-mutable line (Palmer et al., 1998). All three mutants are allelic (Kosslak et al., 1996). The mutants exhibit, under axenic conditions, a progressive necrosis of the root system that can be visualized 5 to 7 days after germination.

The necrotic root mutants are not linked to phosphoglucomutase (EC 2.7.5.1), malate dehydrogenase (EC 1.1.1.37). aconitase (EC 4.2.1.3), diaphorase (EC 1.6.4.3), or isocitrate dehydrogenase (EC 1.1.1.42) (Kosslak et al., 1996).

Our objective was to test for linkage between the necrotic root mutants and seed coat peroxidase.

Materials and Methods

Plant material

Previous experiments have identified three recessive, allelic, necrotic root mutants in soybean (Kosslak et al. 1997). These mutations have been designated *Rn m* (Ames 1) = T328H, *Rn m* (Ames 2) = T329H, and *Rn m* (Ames 3) = T330H.

Cross-pollinations were made between each of the three necrotic root mutants and T261 (cultivar Mandarin Ottawa that is *k2 Mdh1- n*). F1 plants were grown at the University of Puerto Rico-Iowa State University Soybean Nursery near Isabela, Puerto Rico. Individual F2 plants were single plant threshed at the Bruner Farm near Ames, Iowa. The following season F3 seed were used for linkage analyses.

Peroxidase assay

Approximately 4 mm^2 of the seed coat was removed and placed in a spot plate. Five drops of 0.5% guaicol solution were added to each sample. After 10 minutes two drops of 0.1% hydrogen peroxide solution were added to each sample. Presence of either "high" or "low" peroxidase was determined. High peroxidase activity is the dominant allele (*Ep*) and low peroxidase activity is the recessive allele (*ep*). The high control used was PI290136 and the low control was Minsoy.

Necrotic root assay

After a portion of the seed coat was removed for the peroxidase assay the seed was then planted on germination paper and placed in a growth chamber. The roots of homozygous necrotic root genotype turn brown 7 to 10 days after germination. Plant tissue browning is caused by the oxidation of phenolic compounds (Kosslak et al. 1997).

Results

Because seed coat is of maternal origin, F3 seed from individual F2 plants were tested for peroxidase. The expected segregation ratio for peroxidase is 3 high : 1 low. In all three crosses of T261 with the three necrotic root mutants, the peroxidase locus exhibited non-significant P values after a Chi-square analysis (Table 1).

The seed from each F2 plant was sampled to determine the *Rn* allele or the *rn* allele. The expected segregation ratio for the necrotic root phenotype is 5 non-necrotic : 1 necrotic. In all three crosses of T261 with the three necrotic root mutants, the necrotic root locus exhibited non-significant P values after Chi-square analysis (Table 2).

Linkage between peroxidase and necrotic root also was investigated. The expected ratio, assuming no linkage, is 15 high peroxidase non-necrotic : 3 high peroxidase necrotic : 5 low peroxidase non-necrotic : 1 low peroxidase necrotic. For each necrotic root mutant, the test for linkage gave non-significant P values after Chi-square analysis (Table 3). The *Ep* locus is not linked to the *Rn* locus.

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Table 1. Peroxidase test of F3 seed from initial cross of three known necrotic root mutants with T261.

Cross	High	Low	$X^2_{(3:1)}$	P
Rn m (Ames 1) x T261	322	88	2.56	0.11
Rn m (Ames 2) x T261	287	84	1.16	0.28
Rn m (Ames 3) x T261	310	81	3.93	0.05

Table 2. Necrotic root test of F3 seed from initial cross of three known necrotic root mutants with T261.

Cross	Non-necrotic	Necrotic	$X^2_{(5:1)}$	P
Rn m (Ames 1) x T261	316	74	1.50	0.22
Rn m (Ames 2) x T261	306	55	0.50	0.50
Rn m (Ames 3) x T261	300	65	0.32	0.57

Table 3. Linkage analysis between peroxidase (*Ep* locus) and necrotic root (*Rn* locus) generated from F3 data.

Cross	*Ep Rn*	*Ep m*	*ep Rn*	*ep m*	$X^2_{(15:3:5:1)}$	P
Rn m (Ames 1) x T261	251	61	65	13	6.86	0.08
Rn m (Ames 2) x T261	242	39	64	16	3.61	0.31
Rn m (Ames 3) x T261	241	49	60	15	4.31	0.23

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QTL in SoyBase: A New Perspective

Introduction

For a species' database, the data are obviously of utmost importance. The manner in which data are displayed, however, is also significant. Our new model for QTL (quantitative trait loci) in SoyBase links not to the locus itself, but rather to an interval around that locus on its linkage group (LG). Implementing this change demonstrated relationships that had previously gone unnoticed. We have constructed composite linkage groups by superimposing various molecular markers from every published soybean QTL study onto the current USDA molecular maps. The various maps were scaled and aligned to the reference USDA maps by comparing the relative positions of anchor and unique markers. Distances between markers were kept internally consistent within a mapping study. One consequence of this method for composite map generation is that the positions of non-anchor markers are only approximate, especially for those markers that fell far from an anchor locus.

Results

1) QTL are not uniformly distributed throughout the soybean genome. This appears to be true not only among LGs, but also within a single LG. Furthermore, this condition exists in several of the populations in which mapping has been done in soybean: Glyine max (A81-356022) x G. soja (PI468916), Minsoy x Noir1, Evans x Peking, and Young x PI416937.

2) QTL for a given trait occur on comparable LGs in different populations. For example, QTL for soybean cyst nematode (SCN) resistance are found on USDA LG A2 or LG G, or on comparable LGs, in 9 populations (Figure 1).

3) Related QTL, such as various seed traits, may be clustered on one LG. LG C2 contains QTL for 8 seed characteristics in 4 populations (Figure 2). Similar associations exist with other LGs and traits.

4) Frequently a large number of seemingly unrelated, agronomically important traits are coincident on a single LG. A total of 18 traits, from 4 populations, are associated with LG L. These diverse traits include seed weight, first flower, R5, pod maturity, reproduction period, hard seed, pod dehiscence, oil, protein, linolenate, leaf ash, chlorimuron ethyl sensitivity, stem diameter, plant height, leaf length, lodging, yield/height, and height/lodging. The populations are G. max (A81-356022) x G. soja (PI468916), Minsoy x Noir1, Young x PI416937, and PI97100 x Coker237. Other LGs with many QTL are D1, E, and M.

5) Genes and QTL for disease resistance are clustered on certain LGs, and are located within limited distances on those LGs. LGs F, G, and J show closely linked resistance genes, resistance gene analogs, nodulation genes, and molecular markers for QTL for a wide variety of populations. LG F contains Rps3 and markers associated with resistance to SCN, soybean mosaic virus, peanut mottle virus, Pseudomonas syringae, Javanese root-knot nematode, peanut root-knot nematode, corn ear worm, and Phytophthora megasperma (Figure 3). LG G carries Rps4 and markers for resistance to SCN, sudden death syndrome, Fe deficiency, Mn toxicity, and southern root knot nematode. LG J has Rj2, Rps2, Rmd, seven resistance gene analogs, and a marker for resistance to SCN (Figure 3). This explains in part why a cultivar resistant to one pathogen may also be resistant to other diseases.

Conclusion

This perspective on data presentation raises possibilities for further analysis of QTL in soybean. Complete references and figures for these QTL are available through our home page (http://129.186.26.94). In addition, all SoyBase data are available from the National Agricultural Library at http://probe.nalusda.gov:8000/plant/aboutsoybase.html or through our home page.

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See http://129.186.26.94 for complete list of references.

LG A2		POPULATIONS							
LOCUS	cM	1	2	3	4	5	6	7	8
K636	0							-	
A256-1	0.9								
OX04-800	1.9							-	
Bng077-2	2.7								
E043V-2	14.9		-	-	-	-			
A085	3	+	-	-	-	-	-		-
A110	2.8								
K400	9.7		-	-	-	-			-
I	6	-	-	-	-	-	+		+
Rhg4	1								
T153-1	2.2								
A486	4.8								-
php05180a	1								
S07a	8						+		
Gmenod2b	7.3								
BLT65	7.8							+	-
Sac3	3.5								-
OW15-400	4.5							+	
OH03-700	11.2							-	
A111	13.1	-						-	-
A136	2.6		-	-	-	-	+		
P003	5.7								
A638	5.2								
A144-2	21.1								
A117	23.3								
B132	11.2								
p28-8	9.4								
A505	20.2								
T036	10.3								
A572	7.8								
Ap	12								

LG G		POPULATIONS								
LOCUS	cM	1	2	3	4	5	6	7	8	9
C006	0		+	+	+	+				
OI03-450	0							+		
B053	0.6		+	+	+	+				
Bng122	1		+	+	+	+		+		
Bng126	2.6		-	-	-	-				
OG13-490	1.8							+		
php05354a	5								+	
php05219a	5.5								-	
K069-1	7.5	+	-	-	-	-			-	
OE02-1000	14.2							-		
OE04-450	6.1							-		
Bng225	1.4									
A112	6.4							-		+
R017	12.1									
Mng273-2	14.1									
A148	21.9									
A020	5.7									
A426-3	2.7									
A584	2.8									
A816	8.6		-	-	-	-				
T036-2	7									
K227	10									
A885	2.8									
K493	1.7									
cs008-1	8.2									
A235-1	13.1									
H3-54	3.5									
Bng069-1	8.5									
Rps4	3.6									
A378	1.2		-	-	-	+				
A681	7									

Figure 1. LG A2, left, and LG G, right, showing QTL for resistance to SCN. Various molecular markers have been superimposed on the USDA map. Distances between markers and order of closely spaced markers are therefore approximate. + indicates that the marker was associated with SCN resistance; - indicates that the marker was tested but not associated. Populations are 1)M85-1430 x M83-15, 2)Evans x PI209332, 3)Evans x PI88788, 4)Evans x PI90763, 5)Evans x Peking, 6)Peking x Essex, 7)Essex x Forrest, 8)PI437654 x BSR101, and 9)Hartwig x Williams82. A detailed figure showing genetic maps and QTL for these LGs can be found at http://129.186.26.94.

LOCUS	cM	Sd No.	Sd Yld	Sd Wt	Sd Fill	1st Flr	R5	Pod Mat	SdOil
A121	0			○					
A122	15.7								
Bng132-2	7.9								
L199a	8.5			○					
A262	1.6			●					
mQ086	4								
A655	27.5								
A338	4.4					❑		❑	
K262	38.9					❑		❑	◇
K255	2.6					❑		❑	◇
A426	2.9	○	○			○❑		○❑	◇
A063-2	7			☆					
B160	5.9								◇
L148	0.4	○	○			○		○	✦
A635	0			★		❑		❑	
A065	7.9		○		○	○	○	○	◇

LOCUS	cM	Sd No.	Sd Yld	Sd Wt	Sd Fill	1st Flr	R5	Pod Mat	SdOil
R045-2	12.1			☆					
L050-1	8.9	○	○			○❑		○❑	✧
Bng164E	5.4								
A109a	6.4	●	●		○	●○		○	
A397	1.9	○	●		●	●❑	●	●❑	✧
Blt029E	8.8	○	○		●	●○	●	○	
K365	0.9	○	○			○■		○■	✧
Satt79	0.4	○	●					○	
Sct028	11.7	○	○			○		○	
C056	15.3	○	○		○	○❑	○	○❑	
K455	3.5			☆		❑		❑	
A676	19.4	○				○❑		○❑	

Figure 2. LG C2 showing QTL for seed characteristics. Various molecular markers have been superimposed on the USDA map. Distances between markers and order of closely spaced markers are therefore approximate. Solid symbols indicate that marker was associated with trait; open symbols indicate that marker was tested but not associated. Populations are ● ○ Minsoy x Noir1, ■ ❑ G. max (A81-356022) x G. soja (PI468916), ★ ☆Young x PI416937, and ✦ ✧ A87296011 x CX103999. A detailed figure showing genetic map and QTL for this LG can be found at http://129.186.26.94.

LG F			LG J		
Locus	cM	Trait	Locus	cM	Trait
Satt030	0		A363-2	0	
K250	16.3		B101	11.7	
K002	61.7		A060	25.5	
G15d	20.5	SCN	A204	11.5	
A186	2.7	Soybean mosaic virus	A450-1	16.1	
		Peanut mottle virus	RGA6d	9.4	Resistance gene analog
		SCN	Sct065	6	
Rps3	10.3	Phytophthora megasperma	RGA3	26.8	Resistance gene analog
R045	3.0	Phytophthora megasperma	RGA5a	9.8	Resistance gene analog
B212	0	Javanese root-knot nemat.	RGA2a	0	Resistance gene analog
		Peanut root-knot nematode	RGA1e	0	Resistance gene analog
		Corn ear worm	K375	6.5	
K644-1	0	Pseudomonas syringae	B032-1	5.1	SCN
A245	14.4		RGA1a	7.3	Resistance gene analog
Bng190	12.3		Rj2	1.5	Nodulation
B1	2.9		Rps2	0	Phytophthora megasperma
B148	17.2		Rmd	1.5	Downy mildew
A566	8.2		RGA1b	1.7	Resistance gene analog
T092	21.6		RGA1c	4	Resistance gene analog
K102-2	12.9		A199-2	1.8	

Figure 3. LG F and LG J displaying closely associated genes and QTL for disease resistance. Various molecular markers have been superimposed on the USDA map. Distances between markers and order of closely spaced markers are therefore approximate. Not all markers are listed in table. A detailed figure showing genetic maps and QTL for these LGs can be found at http://129.186.26.94.

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The Effect of Pre-Selection on Diversity Detection in Exotic Germplasm

Introduction

One concern in evaluating new germplasm collections is how to most efficiently detect diversity. The goal of this study is to determine if use of isozyme data can increase the efficiency of selection of plant introductions, which represent a wide range of variation for molecular genetic (RFLP) diversity.

Materials and Methods

This study investigated molecular diversity in a collection of soybean germplasm from central China. Two different samples sets were selected from this germplasm collection. The first set, selected randomly, consisted of 42 accessions. The second set, consisting of 64 accessions, was selected to attain maximal isozyme diversity. This selection was based on isozyme diversity among nine isozymes.

DNA for each accession was digested with one of five restriction enzymes. These enzymes were the same ones used to make the *G. max* (A81-356022) by *G. soja* (PI # 468.916) USDA-ARS public map (Shoemaker and Olson, 1993). Electrophoresis, Southern transfer and DNA hybridizations were made following the procedures cited in Keim et al. (1990). Both of the sets were probed with 57 probes. Probes used were ones identified as 'core probes' in a study to determine the ancestry of cultivars commonly grown in the United States (Lorenzen et al., 1995). Each of these probes, in combination with a specific enzyme, is associated with at least one mapped locus.

All RFLP patterns were compared to those of the *G. max* and *G. soja* genotypes used in the public map. The percent of fragments observed that were different from those observed in *G. max* and *G. soja* was calculated.

Results and Discussion

The two selection methods were compared for their ability to reveal RFLP alleles. The set of accessions selected on the basis of maximal isozyme diversity showed a greater range of diversity than the set selected at random. All accessions pre-selected on the basis of isozyme diversity produced some fragments which were not seen in *G. max* and *G. soja* . Accessions pre-selected on the basis of isozyme diversity showed five times more RFLP diversity than the accessions selected at random. The effectiveness of isozyme-based pre-selection over random selection is supported by a t-test with a P value greater than 0.001 level of significance.

Conclusion

Pre-selection based on isozyme diversity identified a set of accessions with more variation in RFLP allelic differences than did random selection.

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Table 1. Frequency of rare alleles

Selection method	Maximal isozyme diversity	Random
Range of variation	0.45 - 0.02 = 0.43	0.05 - 0.00 = 0.05
Mean frequency of rare alleles	0.10	0.02
Deviation from mean	0.07	0.01

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Evidence for Microsynteny among Homoeologous Bacterial Artificial Chromosome Clones of Soybean

Introduction

Genetic mapping in plants has demonstrated a remarkable degree of conservation in the presence and order of DNA sequences among taxa, a phenomenon known as synteny. For example, linkage mapping of restriction fragment length polymorphisms (RFLPs) common among soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*) demonstrated a substantial level of macrosynteny, synteny at the level of centimorgan (cM) distances (Boutin et al, 1995). More recently, microsynteny between related organisms has been discovered by looking at specific gene sequences common to corn (*Zea mays*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*) (Chen et al, 1997). In this study, bacterial artificial chromosome (BAC) clones were used to investigate conservation of DNA sequences at the level of kilobase pair (kbp) distances.

DNA marker analysis has demonstrated that soybean is a paleopolyploid, in which long stretches of the genome are found to be duplicated in multiple locations (Shoemaker et al, 1996). Often the organization of the duplications was found to be complex, with segments from several different linkage groups homoeologous with one another, as well as the existence of multiple rearrangements within otherwise colinear segments. Because this earlier study was based on RFLP linkage mapping, it focused on macrosynteny among homoeologous regions of the genome. Through the use of a soybean BAC library, we now present preliminary results indicating that microsynteny also occurs among homoeologous segments of the soybean genome.

Materials and Methods

A BAC library for soybean was constructed with high molecular weight DNA from variety 'Faribault' as described in Danesh et al, 1998. This library consists of approximately 30,000 clones with an average insert size of 120 kbp. The library was screened using RFLP markers and high density colony blot filters (produced with the generous assistance of Dr. Rod Wing, Clemson University) to identify clones located near *rhg1*, a major gene for cyst nematode (*Heterodera glycines*) resistance on molecular linkage group (MLG) 'G'. BAC clones uncovered by this process were also analyzed by pulsed field gel electrophoresis (PFGE) to estimate size, as well as restriction fragment analysis to develop a coarse restriction enzyme map. Polymerase chain reaction (PCR) and plasmid rescue were used to isolate the ends of these BACs (as described in Danesh et al, 1998), and these sequences were then used to re-probe the BAC library. This process uncovered several new BAC clones, including some located elsewhere in the genome and described in detail below.

Common regions among the BAC clones were studied by cross-hybridization. This involved using either entire BAC clones or individual sub-clones derived from a given BAC as probes against Southern blots that consisted of restriction digested DNA of related BACs. Typically, these hybridizations were carried out at two different levels of stringency, medium (1X SSC and 65^{o}C) and low (1X SSC and 55^{o}C). To estimate copy number of restriction fragments derived from BACs, "Reverse Southerns" were performed. They consisted of Southern blots of restriction digested BAC DNA probed with 100 ng of radiolabeled, genomic Faribault DNA. Any fragments that were visible after autoradiography were putatively considered to be high copy sequences.

End- and sub-clones were also placed on a linkage map of soybean by traditional RFLP mapping methods using mapping populations derived from PI 209332 X 'Evans' or 'Peking' X Evans (Concibido et al, 1997).

Results and Discussion

To probe the BAC library, one end-clone and one sub-clone immediately flanking the end of BAC K4, and both previously mapped on MLG-G only 0.2 cM away from *rhg1*, were used to uncover several cross-hybridizing BACs (Danesh et al, 1998). Four of these BACs (G15, F9, N24, and C24) were analyzed in detail. Restriction fragment "fingerprint" analysis coupled with hybridization to a G15 end-clone demonstrated that G15 and F9 were contiguous. Reprobing of the library with the other end-clone from G15 uncovered still another BAC clone (J9) that extended this contig. Altogether, this genomic segment was found to stretch between 160 and 200 kbp. By contrast, N24 and C24 were shown to be distinct and non-contiguous with one another or with G15 or K4.

Sub-clones from these BACs were analyzed by RFLP linkage mapping. To insure that the mapping results were associated with the cloned BAC sequence, restriction fragments that were mapped on genomic DNA were always compared to the corresponding sizes of fragments derived from the BAC clone(s). While K4 has been previously anchored to MLG-G, the J9-G15-F9 contig was mapped to MLG-K and N24 to MLG-L. The linkage group location of C24 is still uncertain. However, in a study of macrosynteny among soybean genomic regions, Shoemaker et al (1996) demonstrated substantial conservation among MLG-J, MLG-K, and MLG-L in this genomic region, so it is possible that the C24 BAC could be located on MLG-J. In fact, a resistance gene analog (RGA) has been isolated from C24 using degenerate PCR primers, and it is known that MLG-J is especially rich in RGA sequences (Kanazin et al, 1996).

Cross-hybridization among these BAC clones demonstrated significant regions of sequence conservation. By comparison of

cross-hybridizing *EcoR*I bands among BAC clones, K4 was found to share at least 11.5 kbp with the J9-G15-F9 contig, 8.5 kbp with N24, and 2.5 kbp with C24. (On BAC clone K4, this conserved region is located terminally, so it is unknown if the conserved region stretches further.) Moreover, at least 20 kbp of low to medium copy sequence (including the segment mentioned above) was conserved between the J9-G15-F9 contig and N24 at low stringency.

Although these results indicate regions of microsynteny among unlinked regions of the soybean genome, this interpretation must be tempered by the possibility that some of the putative homoeologies could be due to medium (as opposed to low) copy genomic sequences. In most cases, copy number of cross-hybridizing bands was estimated only by Reverse Southern analysis.

To further refine our study of microsynteny among these genomic regions, we are extending all four of these BAC (contigs) and developing restriction maps for each. Eventually, we will focus on gene sequences found within the BACs and carry out DNA sequencing to compare genomic relationships among them in detail.

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Protein Kinases, Nucleotide-Binding Sites and Other Resistance Gene Analogs Near *Rhg* 1, A Major Soybean Cyst Nematode Resistance Locus

Introduction

A major soybean cyst nematode (SCN) resistance locus *rhg* 1, has been previously uncovered near the telomere of molecular linkage group G of soybean (Concibido et al, 1997). Using molecular markers, several bacterial artificial chromosome clones (BACs) have been identified that are tightly linked to this resistance gene. In our effort toward positional cloning and gene identification of this major locus, we have analyzed some of the BACs for presence of putative resistance genes. In this article, we report preliminary results obtained from BAC-end sequence data, as well as degenerate PCR amplification with primers designed from conserved P-loop (nucleotide binding sites) and transmembrane sequences from coding regions of disease resistance genes in other plant species (Kanazin et al, 1996).

Materials and Methods

BAC library screening.

Using bulked segregant analysis in two soybean populations from crosses Evans x Peking and Evans x PI 209332, Keim et al (unpublished results) uncover the molecular marker AFLP-170. This marker was used to screen our BAC library by radioactive hybridization as well as by PCR amplification of BAC pools. UM-BAC library high density filters were hybridized with the 110 bp AFLP-170 fragment and the results were confirmed by dot blot hybridization of the selected clones.

Preparation of BAC maxi-preps for PCR amplification and sequencing.

A standard maxi-prep alkaline lysis protocol from 250 ml LB cultures of the positive BACs, was followed by ammonium acetate precipitation and purification using QIAprep spin columns (QIAGEN Inc., CA) to prepare sequence-grade DNA.

Amplification of resistance gene analogs (RGAs).

Following the procedure described by Kanazin et al (1996), primers LM638 (5'-GGIGGIGTIGGIAAIACIAC-3') and LM637 (5'-A(A/G)IGCTA(A/G)IGGIA(A/G)ICC-3') were used in a degenerate PCR reaction using several dilutions of BAC DNA. Controls for amplification of vector or bacterial sequences were included. To confirm the results obtained from the degenerate amplification, eight combinations of class-specific primers (RGA 1-8) designed by Kanazin et al (1996) were subsequently used to amplify UM-BAC-L23. The PCR products were electrophoresed on a 1% agarose gel with molecular weight markers.

BAC end sequencing.

Five hundred nanograms of BAC sequence-grade DNA, and 3.2 picomoles of M13-F or M13-R primers were used in each sequencing reaction. The automated sequencing was done using ABI Prism BigDye Terminator Cycle Sequencing Ready reaction kits from PE Applied Biosystems with the following PCR conditions; 96 degrees for 10 seconds, 50 degrees for 5 seconds, 60 degrees for 4 minutes for 25 cycles. The PCR products were passed through Centri-Sep columns (Princeton Separations) and run in a sequencing gel.

Analysis of BAC end sequences.

The sequences were edited for vector or low quality terminal sequences, analyzed and searched for sequence homology and open reading frames with GRAIL, BLASTX, BLASTN, and GeneWorks ™.

Results and Discussion

The molecular marker AFLP-170 has been mapped 1.4 cM on the proximal side of *rhg* 1. As a result of the BAC library screening with this marker, two BACs were identified: UM-BAC-J6 and UM-BAC-L23. These BAC seem to form a short contig in this region of soybean linkage group G, estimated at 60 Kb. Further characterization and mapping of these clones is in progress, including confirmation of linkage map location.

UM-BAC-L23, was amplified with the RGA degenerate primers described above, and in repeated experiments, a single band, approximately 500 bp in size was observed. The vector of the clone pECS-BAC-4 was used as a control, and did not show amplification. To confirm these results, and rule out the possibility of an artifactual amplification, the class-specific primer combinations RGA1 to RGA-8 were used. Using the purified, diluted BAC as a template, a single 500 bp band was observed consistently with RGA-5 and RGA-8 primers. These results are currently being confirmed by sequencing analysis and hybridization.

Right and left ends of UM-BAC-J6 were successfully sequenced, and 600 bp of sequence data were obtained from each end. Analysis with GeneWorks program showed a large putative open reading frame (150 a.a.) on the right end of this BAC. In analyzing this same sequence, BLASTX and BLASTN found strong sequence homologies to receptor-like protein kinases from several plant species (see Table 1). The putative protein kinase domain is located in the first 250 nucleotides of the sequenced right end. This area showed significant homology to known disease resistance genes like *Pto* (Martin et al, 1993), and several serine-threonine protein kinases from *Arabidopsis thaliana* and *Oryza sativa*, among others.

UM-BAC-J6 left end sequence, when analyzed for putative open reading frames, showed 5 smaller ORFs in 4 different frames. BLASTN results demonstrated regions homologous to protein kinases (APK1 protein kinase gene from *A. thaliana*) and leucine-rich repeat/receptor-like kinases (*O. sativa*). See Table 1.

Conclusion

The region around the major SCN resistance gene, *rhg* 1, has been analyzed for the presence of sequences homologous to known disease resistance genes in other plant species. From our preliminary results, we conclude that this region has a high density of resistance gene-like sequences with motifs such as P-loops (nucleotide binding sites), leucine-rich repeats and protein kinases.

We have also recently identified a contig of BACs that extends toward *rhg* 1 from an RFLP locus 0.2 cM away. These BACs come from the USDA-ISU soybean BAC library (Marek and Shoemaker, 1997) and they also seem to contain RGA sequences between 400-500 bp in length, which have been confirmed by DNA sequencing. These potentially interesting genes are being characterized further at present.

It is also interesting to speculate that the resistance gene homologs found by PCR and sequence analysis on UM-J6 and UM-L23 might correspond to genes involved in resistance to sudden death syndrome (SDS) in soybean, caused by *Fusarium solani*. Lightfoot et al (1997) observed co-inheritance of SCN and SDS resistances in linkage group G, and have identified the map location of the SDS resistance gene near markers of the proximal side of *rhg* 1, which coincides with the predicted location of the sequences presented in this study. Further experiments are needed to confirm the exact location of these BAC clones and the possible relationship with interesting resistance genes on linkage group G of soybean.

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Table 1. Sequence homology of UM-BAC-J6 to database sequences

Sequence	Best homology	BlastX P Score	Other homologies	BlastX P Score
UM-BAC-J6 Right End	Protein kinase APK1.*A. thaliana*	7.9e-21	Tomato-*Pto* Tomato-*Fen* Rice-*TMK* kinase Tobacco NPK-15	1.6e-12 3.4e-11 1.0e-13 9.9e-12
UM-BAC-J6 Left End	ARK2 receptor-like protein kinase. *A.thaliana*	4.1e-13	Rice- *TMK* kinase Tomato -*Pto* Tomato-*Fen* Cabbage S-receptor kinase	5.1e-12 2.1e-10 3.1e-09 8.9e-07

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Recombinant Inbred Populations from the Crosses Minsoy-Archer and Noir I-Archer

Introduction

We previously described a recombinant inbred (RI) line population from the cross of Minsoy and Noir I (Orf et al. 1994). Two additional populations have been developed by crossing Minsoy and Noir I with the cultivar Archer (Cianzio et al. 1991). As noted in many publications, RI populations are valuable resources for the scientific community. The Minsoy-Archer and the Noir I-Archer RI populations along with the original Minsoy-Noir I population should provide researchers with unique opportunities not available elsewhere.

The Minsoy-Archer population consists of more than 230 RIL's which are currently in the F_{11} generation. The Noir I-Archer population consists of more than 240 RIL also in the F_{11} generation. These populations were developed by L. Mansur. The populations have been evaluated for the simply inherited traits flower, seed coat and hilum color; peroxidase activity and the quantitative traits date to flower (R1) and maturity (R8), plant height and lodging; leaf length, width and area, seed size, protein content, oil content and yield. To date 88 RFLP markers and 261 SSR markers have been placed on the Minsoy-Archer population and 103 RFLP markers and 229 SSR markers have been placed on the Noir I-Archer-population.

We believe these populations are a valuable public resource and plan to make seed available to all who are interested in using this information. Seed of the RIL's also will be available to those who wish to collaborate. We do ask that those who receive seed agree to make their data publicly available and share it in a timely fashion in the appropriate manner. Seed requests from U.S. scientists can be directed to J.H. Orf, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108. Seed requests from international scientists can be directed to L.M. Mansur, Mansur Agricultural Services, P.O. Box 520, Los Andes, CHILE. Initially the parents and 50 random RI lines will be sent to researchers so that they can assess genetic variability for the trait of interest.

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Genetic Analysis of Soybean Cyst Nematode Resistance in PI 438489B

Introduction

Soybean accession PI 438489B, a new introduction from China, is resistant to soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN) Races 1, 2, 3, 5, 14 (Rao Arelli and Wilcox, 1996; Diers et al. 1996), 6, and 9 (Rao-Arelli et al. 1992). The inheritance of SCN resistance in this PI line has not been fully studied.

Objective of this study was to determine the inheritance of resistance to SCN Races 1, 2, 3, 5, and 14 in PI 438489B.

Materials and Methods

Two soybean lines, PI 438489B, resistant to SCN Races 1, 2, 3, 5, and 14, and having black seed coat color, and 'Hamilton', susceptible to all known SCN races and having yellow seed-coat color, were crossed to generate F_1 hybrids in 1995. The F_1 plants were grown in Puerto Rico and from them F_2 plants were grown at the Agronomy Research Center, Columbia, MO, to generate F_3 families. Fresh leaf tissues were harvested and lyophilized for DNA isolation. Part of the F_2 seeds were used for SCN bioassays. Approximately 180 F_3 families were harvested in the field. Seed coat color was recorded and scored from yellow to black.

Selected F_3 families and random F_2 plants were tested for resistance to SCN Races 1, 2, 3, 5, and 14 in the greenhouse during 1997 based on the method described by Rao Arelli (1994). A set of host differentials including Peking, PI 88788, PI 90763, 'Pickett-71' were used to identify races according to the criteria of race determination of Golden et al.(1970) and Schmitt and Shannon (1992). The cultivar 'Hutcheson' was the susceptible standard in each evaluation.

The index of parasitism (IP) was used as the criteria to distinguish between resistant and susceptible individuals (Golden et al. 1970; Schmitt and Shannon 1992).

Plant DNA samples were extracted from each individual F_2 population by using the CTAB method (Saghai Maroof et al. 1984). Southern Blotting was conducted as described by Qiu et al. (1997). Five different enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, and *Taq*I were used.

Chi-square (χ^2) tests were used to test the goodness-of-fit between observed and expected ratios from all F_2 and F_3 data. In this study, a particular $F_{2:3}$ family was used in different race bioassays which could be traced back to the same F_2 individual, therefore it was possible to test the relationship of resistance genes that controlled different races. IP data of SCN race screening results were transformed by square root for regression analysis to determine linkage between markers and resistance loci.

Results and Discussion

Data showed both F_2 and $F_{2:3}$ have large ranges of variation for IP (Table 1). However, only a few of the F_2 and $F_{2:3}$ lines showed total resistance to any races, which indicated that SCN resistance might be controlled by multiple genes.

One hundred and twelve F_2 individuals were tested for resistance to Race 1. Fourteen plants were categorized as resistant and 98 susceptible, which fit the three gene, two dominant, one recessive gene model (designated as *Rhg, Rhg, rhg*, χ^2=0.34, P-value between 0.5-0.75) controlling resistance (Table 2). Among 177 $F_{2:3}$ families, 4 were classified as resistant, 62 segregating, and 109 were susceptible, which fit an expected segregation ratio of 1(R):26(Seg):37(S) (χ^2=2.98, 0.10<P<0.25).

Data from Race 2 bioassay showed that four genes (one dominant, three recessive) might be involved in resistance in PI 438489B. One hundred and three F_2 plants were susceptible to Race 2. One hundred and eighty $F_{2:3}$ families fit the expected segregation ratio of 1(R): 82(Seg): 173(S) (χ^2=0.66, 0.50<P<0.75) (Table2).

Similar to Race1, resistance to Race 3 in PI 438489B might be controlled by three genes (two dominant, one recessive). One hundred and five F_2 data fit an expected ratio of 9 (R) :55 (S) with χ^2=0.12, P>0.75. $F_{2:3}$ data confirmed the above conclusions (Table2).

Resistance for Race 5 was hypothesized to be controlled by three independent genes (two dominant and one recessive). Among 124 F_2 plants tested, 11 were resistant and 113 susceptible, which fit an expected ratio of 9:55 with χ^2=2.34. $F_{2:3}$ data supported this conclusion.

F_2 data for Race 14 indicated that it fit either the three gene (*rhg, rhg, rhg*) or four gene (*Rhg, rhg, rhg, rhg)* model (Table 2). However, $F_{2:3}$ data didn't support both hypothesis. There was less segregation but more susceptible $F_{2:3}$ families than expected. Indeed, we found this phenomena for all observations. This is reasonable because we have few individuals in each $F_{2:3}$ family, which may not be enough to represent all possible segregating genotypes. For example, the segregating genotype *Rhgrhg, Rhgrhg, Rhgrhg, Rhgrhg* has 3/256 chance to obtain a resistant progeny according to the four gene hypothesis. Therefore we reclassified $F_{2:3}$ families into two categories, resistant and susceptible. F_3 data supported the 3 genes model (*rhg, rhg, rhg*), and fit the 1(R):63(S) expected ratio with χ^2=0.77 (0.5<P<0.75).

From these results, we concluded that resistance to different races in PI 438489B had similar patterns. However, we still don't know the relationship among these resistant genes. Because the $F_{2:3}$ data for all five races came from the same F_2 individual, we calculated the correlation among all five races and with seed coat color (Table3).

Data for all five races were highly correlated with each other (P<0.0001), which indicated that they shared some common resistance genes. Among all the $F_{2:3}$ families, lines 27, 79, 151, and 161 showed resistance to all five races, which included approximately 50% of all resistant families (data not shown). Seed coat color was significantly correlated with Race 1 and 3 but not significantly correlated with Races 2, 5, and 14 in this study.

One hundred fifty eight RFLP markers (Biogenetic Services Inc., Brookings, SD) were used to screen two parents, 32 were polymorphic. Regression analysis showed that four markers, K493, A381, A078, and B072, were significantly related with SCN loci ($P<0.05$) (Table 4).

Three markers, K493, A381, and B072, were related with Race 1 resistance. One marker related to Race 3, 5, and 14 respectively. Probe A381 showed significant correlation with Race 1, 3, and 5 resistance. No markers were found significantly related with Race 2 and seed coat color.

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Table 1. Mean and range of IP among F_2 and $F_{2:3}$ Population.

SCN Races And Generations	Total Number of Plants	IP Mean and Range: Resistant IP	Resistant IP Range	Segregation IP	Segregation IP Range	Susceptible IP	Susceptible IP Range
Race 1							
F_2	112	2.14	0-8.05	-	-	156.11	21.48-383.89
$F_{2:3}$	177	1.40	0.41-3.15	47.62	4.37-114.43	83.50	24.89-210.63
Race 2							
F_2	103	-	-	-	-	73.83	20.42-380.89
$F_{2:3}$	180	1.69	0.00-6.86	38.30	8.57-99.63	57.24	27.14-102.86
Race 3							
F_2	105	2.41	0.00-9.15	-	-	64.65	12.73-251.63
$F_{2:3}$	180	1.76	0.35-6.95	26.82	4.34-75.44	59.80	22.05-141.42
Race 5							
F_2	124	2.58	0.00-9.45	-	-	84.63	14.17-231.50
$F_{2:3}$	178	1.64	0.64-4.35	23.81	5.98-74.75	40.59	13.49-85.18
Race 14							
F_2	98	2.58	-	-	-	84.63	17.08-125.36
$F_{2:3}$	166	5.66	2.24-8.95	28.58	11.47-67.13	56.17	21.26-112.26

Table 2. Reactions of F_2s, and $F_{2:3}$ Families to SCN Races 1, 2, 3, 5, and 14 and Genetic Analysis.

SCN Races & Generations	Number of Plants: R	Seg	S	Hypothesized Resistant Genes	Expected Genetic Ratio	$\chi 2$	P
Race 1							
F_2	14	-	98	*Rhg, Rhg, rhg*	9:55	0.34	0.50-0.75
				Rhg, rhg, rhg	3:61	13.77	<0.01
$F_{2:3}$	4	62	109		1:26:37	2.98	0.10-0.25
Race 2							
F_2	0	-	103	*Rhg, rhg, rhg, rgh*	3:253	0.42	0.50-0.75
$F_{2:3}$	1	53	126		1:82:173	0.66	0.50-0.75
Race 3							
F_2	17	-	88	*Rhg, Rhg, rhg*	9:55	0.12	0.50-0.75
				Rhg, rhg, rgh	3:61	28.60	<0.01
$F_{2:3}$	6	69	105		1:26:37	3.41	0.10-0.25
Race 5							
F_2	11	-	113	*Rhg, Rhg, rhg*	9:55	2.34	0.25-0.50
				Rhg, rhg, rhg	3:61	3.97	0.025-0.05
$F_{2:3}$	5	74	99		1:26:37	1.95	0.25-0.50
Race 14							
F_2	1	-	95	*Rhg, rhg, rhg, rgh*	3:253	0.17	0.50-0.75
				rhg, rgh, rgh	1:63	0.17	0.50-0.70
$F_{2:3}$	4	49	113	*Rhg, rhg, rhg, rgh*	1:82:173	17.60	<0.01
				rhg, rgh, rgh	1:26:37	8.83	0.01-0.025

Table 3. The Correlation Between IP For Five Races in $F_{2:3}$ Families and Seed Coat Color[a]

	Race 1	Race 2	Race 3	Race 5	Race 14
Race 2	0.3909***[b]				
Race 3	0.4241***	0.3175***			
Race 5	0.3449***	0.4236***	0.3133***		
Race 14	0.3050***	0.3448***	0.3048***	0.3900***	
Seed Coat Color	-0.2713**	-0.1035	-0.2800***	-0.1390	-0.0501

Note: a. Seed coat color in F2:3 families were classified as: 1=yellow, 2=buff, 3=reddish brown, and 4=black.

b. *** and ** significant at the 0.0001 and 0.001 probability levels, respectively.

Table 4. RFLP markers related to SCN races 1, 3, 5, and 14 resistant loci.

Races	Markers	F-Value	P-Value	R^2-Value(%)	# of Samples
Race 1	K493	3.41	0.035	3.95	168
	A381	2.94	0.046	3.92	147
	B072	5.10	0.007	7.32	132
Race 3	A381	3.24	0.041	4.22	150
Race 5	A381	3.24	0.042	4.28	148
Race 14	A078	7.44	0.007	4.85	148

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Genetic Relationships among Soybean Plant Introductions with Resistance to *Heterodera Glycines* Using Rflp's

Introduction

In the USA, soybean cyst nematode (*Heterodera glycines* Ichinohe=SCN) has been one of the most destructive pests of soybean (*Glycine max* (L.) Merr). This nematode reduces the production of soybean and causes enormous economic losses to growers each year (Qiu et al. 1997). Presently, the best way to control this pest is to plant SCN resistance cultivars. This is most economical and safe to the environment.

Plant introductions have been tested for SCN resistance and several resistant PIs have been identified (Ross and Brim, 1957; Young, 1990; Nelson et al. 1994; Arelli et al., 1997). However, very few have been used in the breeding of SCN resistant cultivars. Breeders need to increase the diversity of source of SCN resistance genes because the nematodes may easily overcome resistance currently available in existing soybean cultivars.

The information of genetic relationships between and among species is very important to crop breeding programs. However, because the information on pedigrees is not available and traditional techniques cannot determine genetic distances among SCN resistance introductions, molecular methods are the most useful (Diers et al. 1997; Prabhu et al. 1997). Recently, thirty-three additional PIs from soybean germplasm collections were found to be resistant to SCN Races 1, 2, 3, 5, or 14, (Nelson et al. 1994; Arelli et al. 1997) however, the information on their genetic relationships is not available.

Objectives of this research were to determine genetic relationships using RFLPs among thirty-three PIs, based on the genetic distances and to identify unique PIs that are not closely related to Peking, PI88788 and PI437654 or 'Hartwig'.

Materials and Methods

Fifty soybean genotypes included thirty-three newly identified SCN resistant PI lines (PI567285 through PI567660B), eleven known resistant genotypes and six susceptible controls were analyzed for their genetic relationships using RFLPs. Seeds were provided by Dr. Randy L. Nelson, Curator, Soybean germplasm collection, USDA-ARS, Champaign, Illinois.

Genomic DNA isolation restriction enzyme digests, electrophoresis, and southern blotting were conducted according to established methods. Probes for RFLP analysis were developed by Dr. Randy Shoemaker, Iowa State University-USDA, ARS, and were purchased from Biogenetic Services, Inc. (Brookings, SD). Each polymorphic band was scored according to Zhang et al. (1996). The dendrogram was constructed by Ward's method (SAS Institute Inc., Cary, NC 1990).

Results and Discussion

Maturity group, seed color and SCN reaction of soybean genotypes used in this report are presented in Table 1.

Out of 109 RFLP probes, 78 probes showed polymorphism. Three hundred-twenty-four polymorphic fragments were scored and were used in cluster analysis.

The cluster analysis placed the genotypes into two major groups (Fig 1). In the upper part of the dendrogram, there was a major group (II) of SCN resistant PIs distant from previously used sources of resistance with the exception of susceptible 'Minsoy'. In this major group, seventeen SCN resistant PIs were included and eleven were yellow seeded. Among the yellow seeded PI lines, 567364, 567373A and 567445B, were resistant to multiple SCN Races.

The lower part, i.e major group (III), had several subgroups and included were Southern cultivars that were either resistant or susceptible to SCN together with PI90763, PI88788, PI437654, and 'Peking.'

The subgroup numbers 1 through 4 included Northern elite cultivars, 'Kenwood', 'BSR101', 'Dunfield' that was susceptible to SCN together with several SCN resistant PIs including PI209332. 'Pickett-71', a southern cultivar with SCN resistance was the exception.

There was some association between the resistance of PIs and their grouping pattern. Some PIs that were closely related had similar resistance reactions. For example, PI567516C and PI567342 were closely grouped, green seeded, and had nearly similar resistance reactions. It is therefore likely that grouped lines may have resistance genes in common. However, allelism tests or genetic mapping studies alone may conclude whether these PIs have resistance genes in common or at different loci.

In this study, our results showed that there are PIs that have high levels of SCN resistance and are genetically distant from previously used sources. These PIs could therefore potentially have new SCN resistance alleles and may provide durable resistance in future soybean cultivars.

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Table 1. Maturity group, seed color, and reaction to SCN for 50 soybean genotypes.

Genotype	Maturity group	Seed color	SCN Reaction[1]				
			1	2	3	5	14
PI567285	IV	yellow	S	S	R	S	S
PI567286	IV	yellow	S	NA	R	S	R
PI567303A	IV	black	S	NA	S	S	R
PI567325	V	yellow	S	S	R	S	S
PI567328	V	yellow	S	S	S	S	R
PI567336A	IV	black	S	MR	R	R	S
PI567336B	IV	black	MS	MR	R	R	S
PI567342	V	green	MR	R	R	R	S
PI567363*B	III	yellow	S	S	R	S	S
PI567364*	II	yellow	S	S	R	MS	R
PI567365*	III	green	S	S	R	MS	S
PI567373*A	IV	yellow	S	S	S	MR	R
PI567373B	V	yellow	MS	MS	S	MS	R
PI567400	V	yellow	S	S	S	S	R
PI567415*A	IV	yellow	MR	MS	S	MR	R
PI567418A	II	yellow	S	S	S	S	R
PI567421	IV	yellow	S	S	S	S	R
PI567445*B	IV	yellow	MR	MS	S	NA	R
PI567491A	III	black	R	MR	R	R	S
PI567492	IV	yellow	S	S	S	S	R
PI567507B*	II	yellow	S	S	S	S	R
PI567510A	III	yellow	S	S	S	S	R
PI567512B*	II	yellow	S	S	S	S	R
PI567516C	IV	green	R	MR	R	S	S
PI567535A	IV	yellow	S	S	S	NA	R
PI567562A	IV	yellow	S	S	R	S	S
PI567568A	IV	yellow	MS	S	R	S	R
PI567577	IV	yellow	S	S	R	S	S
PI567581	IV	yellow	S	S	S	S	R
PI567583*C	IV	yellow	MS	S	S	S	R
PI567583D	IV	yellow	S	S	R	S	S
PI567636	IV	yellow	S	S	R	MS	S
PI567660B*	V	yellow	S	MS	R	S	S
BSR101	I	yellow	S	S	S	S	S
Dunfield	III	yellow	S	S	S	S	S
ESSEX	V	yellow	S	S	S	S	S
Forrest	V	yellow	R	S	R	S	S
Hartwig	V	yellow	R	R	R	R	R
Hutcheson	V	yellow	S	S	S	S	S
Kenwood	II	yellow	S	S	S	S	S
Minsoy	0	yellow	S	S	S	S	S
Peking	IV	black	R	S	R	R	MS
Pickett-71	VI	yellow	R	S	R	S	S
PI88788	III	black	S	S	R	S	R
PI90763	IV	black	R	R	R	R	MS
PI209332	IV	black	MS	S	R		R
PI437654	III	black	R	R	R	R	R
PI438489B	IV	black	R	R	R	R	R
PI467312	II	green	S	MS	R	R	R
PI507354	I	yellow	R	MR	R	R	M

1. R=Resistant; MR=Moderately Resistant; MS=Moderately Susceptible; S- Susceptible

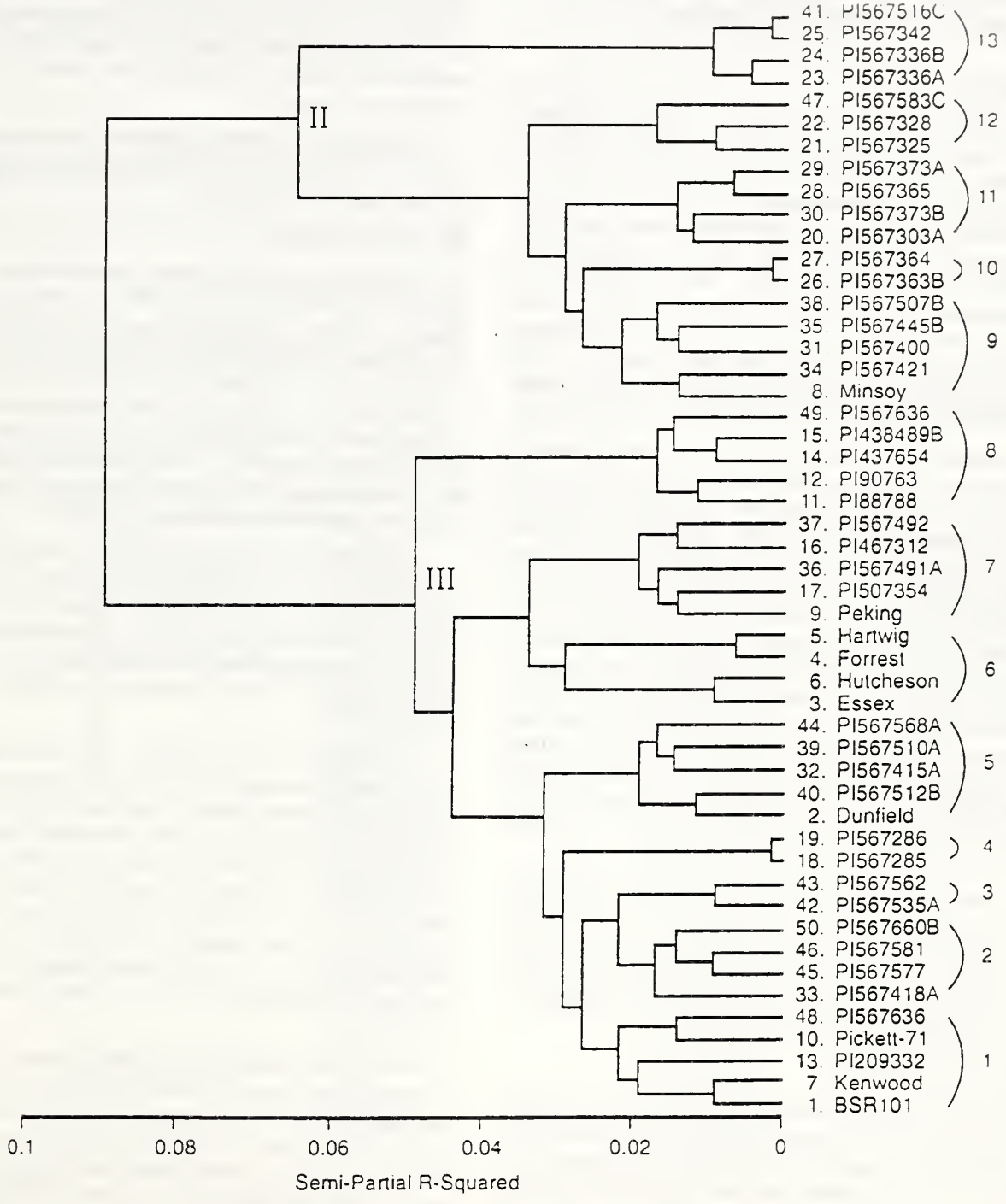

Fig. 1. Clustering of 50 soybean SCN resistant and susceptible lines using RFLPs.

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Harvest Index as a Tool to Combine High Yield and High Protein Content in Soybean

Introduction

The soybean, often called "The Miracle Crop", is an excellent source of protein (Mounts et al., 1987; Fulmer, 1988) and has the potential to supply adequate and nutritious food and feed for use by ever-increasing world population. This potential, however, is limited due to a negative correlation between soybean seed yield and protein content (Burton, 1988; Leffel and Rhodes, 1993; Serretti et al., 1994; Pantalone et al., 1996). Current soybean cultivars average approximately 41% protein and 21% oil in the seed, on a dry weight basis (Leffel and Rhodes, 1993). In order to meet food needs of increasing world population, world soybean production will need to increase to meet the projected demand for high quality protein (Pantalone et al., 1996).

Harvest index, a measure of biological efficiency defined as ratio between economic product (seed) and the total biomass, is positively correlated to soybean yield (Schapaugh and Wilcox, 1980; Bhardwaj and Bhagsari, 1989; Bhardwaj and Bhagsari, 1990; Bhardwaj and Bhagsari, 1991; Dadson et. al., 1990). Two avenues relative to harvest index have been suggested for increasing crop yields. Austin et. al., (1980) advocated reallocation of assimilates targeted for stem and leaf-sheath dry matter production to ear dry matter in cereals. They calculated that a reduction in stem and leaf sheath dry matter to half current values and a reallocation of this dry matter to the ear could raise the harvest index to about 0.62 from 0.50 assuming a constant biomass yield. On the other hand, Hays and Walker (1984) postulated that increases in total dry matter production, while maintaining the current harvest indices might provide the most likely route to further increases in yield.

Despite the results indicating a positive relationship between yield and harvest index, breeding for increased biological efficiency as a tool to increase the yield has not been employed in soybean. The objective of this research was to determine if harvest index can be used as a tool to select high yielding lines from high protein breeding populations.

Materials and Methods

The base breeding material consisted of 28 F_6 soybean populations. These populations were derived by crossing high protein breeding lines whose protein content varied from 45 to 52 percent. The F_6 material was grown at Randolph Farm of Virginia State University located in Ettrick, Virginia (approximately 37^0-15' N and 077^0-30.8'W) during 1995. The apparent harvest index was recorded for 1264 single plants. In this field planting, six popular soybean cultivars (Asgrow 4341, Asgrow 5843, Chesapeake, DP-415, Holladay, and Hutcheson) were included as checks. Data on harvest index (weight of seed/total weight including stems, pods, and seed at final harvest), seed yield, and protein content were recorded on 1264 single plants. The protein content was determined at National Center for Agricultural Utilization Research, Peoria, Illinois, using NMR technology.

Based on these data, 15 families (five each with high, medium, or low mean harvest index) were selected for further evaluation. Within each selected family, 15 progenies were selected (five each with high, medium, or low harvest index). Comparisons of various traits from these evaluations were used to determine if harvest index can be used as an indirect selection tool to develop soybean lines that combine high yield with high protein content.

All data were analyzed using analysis of variance and other procedures in SAS (SAS, 1996). A 5% level of significance was used for all statistical comparisons.

Results and Discussion

The harvest index, protein content, and seed yield of 28 populations varied from 31.5 to 42.0 percent, 47.1 to 54.7 percent, and 1380 to 2838 kg/ha (Table 1). In comparison the harvest index, protein content, and seed yield of six cultivars varied from 35.4 to 44.8 percent, 40.3 to 43.9 percent, and 1405 to 2524 kg/ha. The means for harvest index, protein content, and seed yield for six cultivars were 40.5 percent, 42.4 percent, and 1967 kg/ha, respectively. These comparisons indicated that the 28 segregating populations had higher protein content than the six soybean cultivars that are grown in Virginia.

Within 28 populations, the progenies also varied for different traits (Table 1). On an individual progeny basis, the harvest index varied from 11 to 63 percent, the protein content varied from 42 to 62 percent, and seed yield varied from 684 to 4682 kg/ha. These data indicate that considerable variation existed among this breeding material for harvest index, protein content, and seed yield.

A comparison of high, medium, and low harvest index groups (Table 2) indicated that grouping of soybean lines based on harvest index was successful. Significant differences existed among the three groups for harvest index, protein content, and seed yield. The high harvest group had significantly higher harvest index, and seed yield as compared to the low harvest index group. It was interesting to observe that the protein content of high and medium harvest index groups was statistically similar (51 and 50 percent, respectively and was considerably greater than that of six soybean cultivars (42.4 percent).

An analysis of correlation relationship between various traits indicated that grouping of 1264 progenies into high, medium, or low harvest index groups caused a change in relationships between various traits. The correlation between seed yield and protein in six cultivars and that among low harvest index group was negative and significant (-0.36 and -0.32, respectively) whereas this correlation in the high and medium harvest index groups was not significant (-0.19 and -0.21, respectively). This indicates that selection of high harvest index lines has changed the relationship to facilitate selection of high protein and high yielding soybean lines.

The results reported here indicate that indirect selection for high yield and high protein content using harvest index as a selection tool has potential. Additionally, the change in correlation coefficients between seed yield and protein content caused by selection based on harvest index, imply that biological efficiency is an important aspect of crop growth and should be considered in breeding programs. These results indicated selection in high-protein soybean breeding material, using harvest index as a selection tool, was successful in identifying soybean progenies that combined high seed yield with high protein content.

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Table 1: Mean and ranges for various traits in 28 soybean populations grown during 1995 in Virginia.

Population Number	Number of Progenies	Harvest Index(%)	Seed Protein(%)	Yield Kg/ha
VX25	55	36.6(25.9-46.8)	50.2(45.3-54.2)	1715(1014-2758)
VX26	57	36.6(22.5-45.9)	52.8(47.0-61.9)	1920(1154-3728)
VX27	58	37.9(28.3-48.1)	49.5(42.5-56.4)	2330(1146-4379)
VX28	59	38.3(23.7-55.0)	50.3(45.6-55.7)	1919(1097-3688)
VX29	44	37.2(26.6-63.1)	51.6(43.9-56.4)	2111(820-3295)
VX30	48	36.6(25.4-47.0)	51.8(44.1-57.1)	2445(1301-4682)
VX46	49	33.0(23.0-41.3)	54.7(49.2-60.8)	2054(911-4363)
VX47	48	34.2(18.1-46.2)	53.0(43.5-61.0)	2377(1194-4662)
VX48	45	31.5(11.4-48.0)	53.8(47.8-58.5)	1770(684-3251)
VX49	48	33.8(13.8-44.2)	52.8(46.7-58.1)	2209(732-3995)
VX50	41	35.6(24.7-43.0)	52.1(42.9-55.3)	1943(905-3863)
VX52	41	36.1(27.9-45.9)	52.1(49.0-58.0)	2190(816-4184)
VX53	45	36.4(23.8-59.2)	51.4(48.0-54.8)	2025(1111-4673)
VX54	46	36.3(22.7-45.4)	50.8(42.8-55.2)	2328(1178-4416)
VX55	31	36.8(28.5-46.9)	51.1(47.3-56.1)	2459(1161-4448)
VX56	34	40.1(28.5-49.2)	50.5(46.6-55.8)	2340(1103-3576)
VX57	48	41.1(25.8-54.4)	50.6(46.7-54.8)	2342(1223-4257)
VX58	50	37.7(23.8-47.9)	50.4(42.7-54.7)	2549(1561-4568)
VX59	47	38.8(30.3-48.7)	50.3(42.9-54.5)	2510(1447-4522)
VX60	48	41.9(27.6-48.4)	49.2(44.7-52.4)	2291(1213-3880)
VX61	49	40.5(30.6-49.1)	49.5(45.6-53.7)	2682(1597-4586)
VX62	51	42.0(28.6-52.5)	48.7(42.6-52.5)	2838(1319-4633)
VX63	48	39.9(29.8-48.8)	50.1(44.7-53.8)	1907(729-4066)
VX64	50	41.3(28.0-50.6)	49.6(43.0-53.9)	2085(1159-2968)
VX65	50	42.0(32.8-51.7)	49.2(43.0-53.9)	2307(1439-4041)
VX66	35	40.2(31.2-51.0)	49.7(44.1-54.7)	2153(977-3379)
VX67	27	36.7(30.2-43.9)	47.1(43.0-51.3)	1706(898-3236)
VX68	12	31.7(25.0-43.5)	49.3(43.3-54.2)	1380(637-2752)
Soybean Cultivars				
Asgrow 4341		35.4	42.4	1420
Asgrow 5843		41.3	42.9	2009
Chesapeake		39.0	43.9	1405
DP 415		45.0	43.3	2579
Holladay		37.6	40.3	1863
Hutcheson		44.8	41.7	2524
Mean		40.5	42.4	1967

Table 2: Comparison of soybean progenies with low, medium, and high harvest index values

Harvest Index	Harvest Protein Index(%)	Yield %	Kg/ha
Low	29.6 C[x]	51.9 A	1776 C
Medium	37.3 B	51.0 AB	2175 B
High	45.5 A	50.2 B	2757 A
Six Cultivar Mean	40.5	42.4	1967

x: Means followed by similar letters are not different according to Duncan's Multiple Range Test (5% level).

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Response of Soybean Breeding Lines to Corn Earworm Leaf Damage

Introduction

Corn earworm (CEW), *Helicoverpa zea* Boddie is the most serious pest of soybean in much of the mid-atlantic and southern coastal plain (Stinner et al., 1980). The moth is attracted to soybean at the time of flowering (Johnson et al., 1975) where it prefers to oviposite on developing new foliage (Eckel et al., 1992). Small larvae are found in even greater density in young foliage, perhaps because of production provided by rolled leaves (kraemer, et al.,1997). Even though insecticides provide adequate control of CEW, the use of host plant resistance is economically and ecologically superior management techniques. The objectives of this study were to: determine the magnitude of genotype x year interaction of CEW larvae weight, and identify breeding lines resistant to CEW damage.

Materials and Methods

A total of 35 soybean lines, 24 from Virginia State University (VSU), and six from Dr. Glenn Buss, Virginia Polytechnic and State University (VPI & SU) and four lines from Drs. Tammy Carter and Joe Burton, ARS/USDA at North Carolina State University were used for this study. Three replications of each genotype were planted in single-row plot, in a randomized complete block design, on 30 May 1996 and 29 May 1997 at Randolph Research Farm of VSU, Petersburg, Virginia. Each plot was 1.8 m long, with a spacing of 1.8 m between rows and a seeding rate of 28 seeds m^{-1}.

Foliage was collected when plants had reached the flowering stage. The trifoliolates were separated into leaflets with petioles removed and placed in 150 x 15 mm plastic petri dish lined with moistened Whatman #2 filter paper. Four petri dishes were used per genotype per replication, there were a total of 12 petri dishes per genotype. Two neonate CEW larvae were placed on the foliage in each petri dish to allow for possible first instar mortality unrelated to leaf antibiosis. The petri dishes were then held in an environmental chamber at 25°C and 14:10 (L:D:) photoperiod. Although, relative humidity within the environmental chamber was not controlled (50-60%) the filter paper within each petri dish was kept moist with approximately 1 ml of water every other day. The number of larvae per petri dish was reduced to one after 4 to 5 days. After 10 days the larvae were weighed and mortality was determined. When more than one CEW larvae was found in a petri dish, probably from eggs brought on the foliage. The largest larvae was selected for analysis.

Analyses of variance and variance component estimations were performed. Genotype was considered as fixed and year as a random effect. Variance components were calculated by equating appropriate mean squares for their expectations and solving for the components. Heritability was estimated from the mixed model and was calculated from Table 1 as described by Milligan et al. (1990)]: $h^2 = Q^2_g/(Q^2_g + Q^2_{gy}/_y + Q^2_e/_{ry})$. The terms Q^2_g, Q^2_{gy}, and Q^2_e refer to estimates of genotype, genotype x year, and pooled error term, respectively. The divisors y, and r refer to number of years and replications, respectively.

Results and Discussion

Highly significant ($p < 0.01$) difference for CEW larval weights were observed among genotypes (Table 1). The significant genotypic differences indicated that genetic variation exists for CEW resistance among the tested genotypes. This offers promise for selection and improvement through hybridization. Moreover, a significant genotype x year interaction (GYI) was present. This interaction was the result of a change in the magnitude of the differences between the genotypes in the different years. The significant GYI observed suggests that the performance or response of the genetic materials used in this study were not stable from one growing season to another. Stable genotype is defined as the ability of a genotype to avoid substantial fluctuation in leaf defoliation over a range of environments, a breeding objective that is difficult to achieve. Stability could be due to physiological, morphological, and/or phenological mechanism.

Determining the genotype x environment interaction (GEI) is common in plant breeding programs and germplasm evaluation trials because, it is often desirable to find genotypes that show little interaction with the environment. Those genotypes may be regarded as stable. Our results indicate that the CEW resistance of these genotypes differ from one growing season to another. Selections among these genotypes for CEW resistant would be different in each growing season. This result suggests that GYI must be considered when breeding for CEW, because this interaction affects both the rank and magnitude of the tested genotypes.

The sum of squares of the sources of variation expressed as percentages revealed the relative contribution of each source to the total variance. The pooled error contributed proportionally the highest (55.20 %) to the total variability, followed by replication (year) and genotype with 27.16 %, and 11.24 %, respectively. The proportional contribution of year to the total variation was only 1.92 %. The GYI contributed 4.53% to the total variation. Among the major components, the interaction component contributed the least. The pooled error sum of square on the other hand contributed proportionally more than genotype and GYI.

In this study, the two-year heritability estimate for CEW resistance was 59.67%. As indicated by a moderate heritability estimate, selection of genotypes for CEW resistance among the tested genotypes appear to be effective. Highly heritable characters are important to plant breeders as they allow selection to be based reliably on the phenotypic performance.

Breeders often make selections based on the ranking of genotypes in one or more environments. Consequently, the impact of GYI on the ranking of genotype in different year is of interest. The rank order of CEW larvae weight of genotypes tested at different growth years is presented in Table 2. The mean weight of CEW larval reared on foliage was 150 mg in 1996 and ranged from 62 to 214 mg and was 140 mg in 1997, ranged from 63 to 180 mg. The susceptible genotypes Essex and PI 399 055 had 148 and 179 mg mean larval weights, respectively, which were significantly higher than L76 0049, a

resistant check. The resistant check had 111 and 92 mg larval weights, respectively, for the 1996 and 1997 seasons.

A comparison of genotypes at different years showed that mean CEW larval weights can be categorized into three groups. In 1996 out of 35 lines tested 34% showed mean CEW larval weight lower than overall genotypic mean, 26% have equal to the overall mean and about 40% lines have higher values than the overall mean. Those breeding lines which exhibited low CEW larval mean weight values were: VS94-01, VS94-01, VS94-11, VS94-18, VS94-19, VS94-21, VS94-24, VS94-26, V89-2614, V91-0623, and N91-8005.

The 1997 results were similar to those of 1996 in that 37 % had lower values than the overall mean, 26% were equal to the overall mean, and 37 % had higher values than the overall mean. In 1997, lines that registered with low CEW larval weight mean values were: VS94-05, VS94-07, VS94-11, VS94-12, VS94-19, VS94-26, VS94-44, VS94-45, V89-3426, V91-0623, N90-7199, and N91-8005.

An overall evaluation of genotypic CEW larval weights over both years found about 29% with lower values than the overall mean, 23% with equal values to the mean and the largest group about 49 % lines have higher than the overall mean. Over two growing seasons five lines, VS94-11, VS94-12, VS94-19, VS94-26, and N91-8005 consistently showed lower CEW mean larval weight values. Thus, these results suggested that the expression of CEW resistance in these five lines are relatively less affected by environmental fluctuations and they could serve as genetic source in breeding program, aimed at reducing CEW larval foliage damage in soybean genotypes. In each of the two growing seasons and the overall average results indicated that the susceptible checks, Essex and PI 399055 exhibited higher values than the overall genotypic means. While, the resistant check, L76 0049 registered lower than the overall genotypic means. These results demonstrated that the petri dish essay technique was reliable enough to separate the susceptible from the resistant genotypes. Moreover, these results suggested that sufficient genetic variation exists among genotypes to make improvement through selection.

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Table 1. Combined analysis of variance across years and genotypes.

Source of Variation	df	Sum of squares	Percentage¶ contribution	Mean square	Variance component
Year (Y)	1	0.09134	01.92	0.0913	
Rep[Y]	1	1.28893	27.16	0.3223	
Genotype (G)	34	0.53334	11.24	0.01568**	0.00156
G x Y	34	0.215091	04.53	0.0063**	0.00091
Pooled error	728	2.619962	55.20	0.0036	0.00360

¶ - Percentage contribution to the total variance
** - Significant at 1 % probability level

Table 2. Mean Corn earworm larval weight (mg) with ranking of thirty-five soybean breeding lines at averaged over years and replications.

Genotype	1996	Rank	1997	Rank	Overall[a]	Rank
VS94-01	143	23	161	05	152	13
VS94-02	172	09	142	12	158	09
VS94-04	156	19	131	17	144	16
VS94-05	147	22	096	31	121	29
VS94-07	157	18	119	24	137	20
VS94-08	186	05	144	10	165	06
VS94-11	093	33	089	33	091	34
VS94-12	062	35	063	35	062	35
VS94-13	162	13	155	08	159	07
VS94-16	113	30	137	14	125	26
VS94-17	160	15	127	19	142	17
VS94-18	136	24	159	07	148	14
VS94-19	126	27	116	25	122	28
VS94-20	160	16	149	09	155	11
VS94-21	136	34	132	16	106	32
VS94-22	176	07	141	13	159	08
VS94-24	121	28	161	04	141	18
VS94-26	133	26	115	26	124	27
VS94-27	182	06	166	03	174	05
VS94-42	193	04	160	06	176	03
VS94-44	169	10	103	29	134	24
VS94-45	167	11	100	30	133	24
V89-2614	119	29	123	22	121	30
V89-2623	194	03	180	01	187	01
V89-3426	155	21	115	27	134	22
V91-0613	159	17	071	34	133	23
V91-0623	133	25	123	21	129	25
V91-1002	174	08	176	02	175	04
N90-7199	200	02	111	28	156	10
N90-7202	162	14	143	11	152	12
N91-8005	107	32	126	20	116	31
N93-1128	156	20	122	23	139	19
ESSEX	167	12	131	18	148	15
PI 399055	214	01	131	15	179	02
Mean	**150**	-	**130**	-	**140**	-
$LSD_{(0.05)}$	044	-	055	-	035	-

[a] corn earworm larval weight in mg averaged over two years and three replications

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